

THE UNIVERSITY OF LIVERPOOL

ANNALS  
OF  
TROPICAL MEDICINE AND  
PARASITOLOGY

ISSUED BY THE  
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR J. W. W. STEPHENS, M.D.Cantab., F.R.S.

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VOLUME XVI

(March 31, 1922, to December 30, 1922)

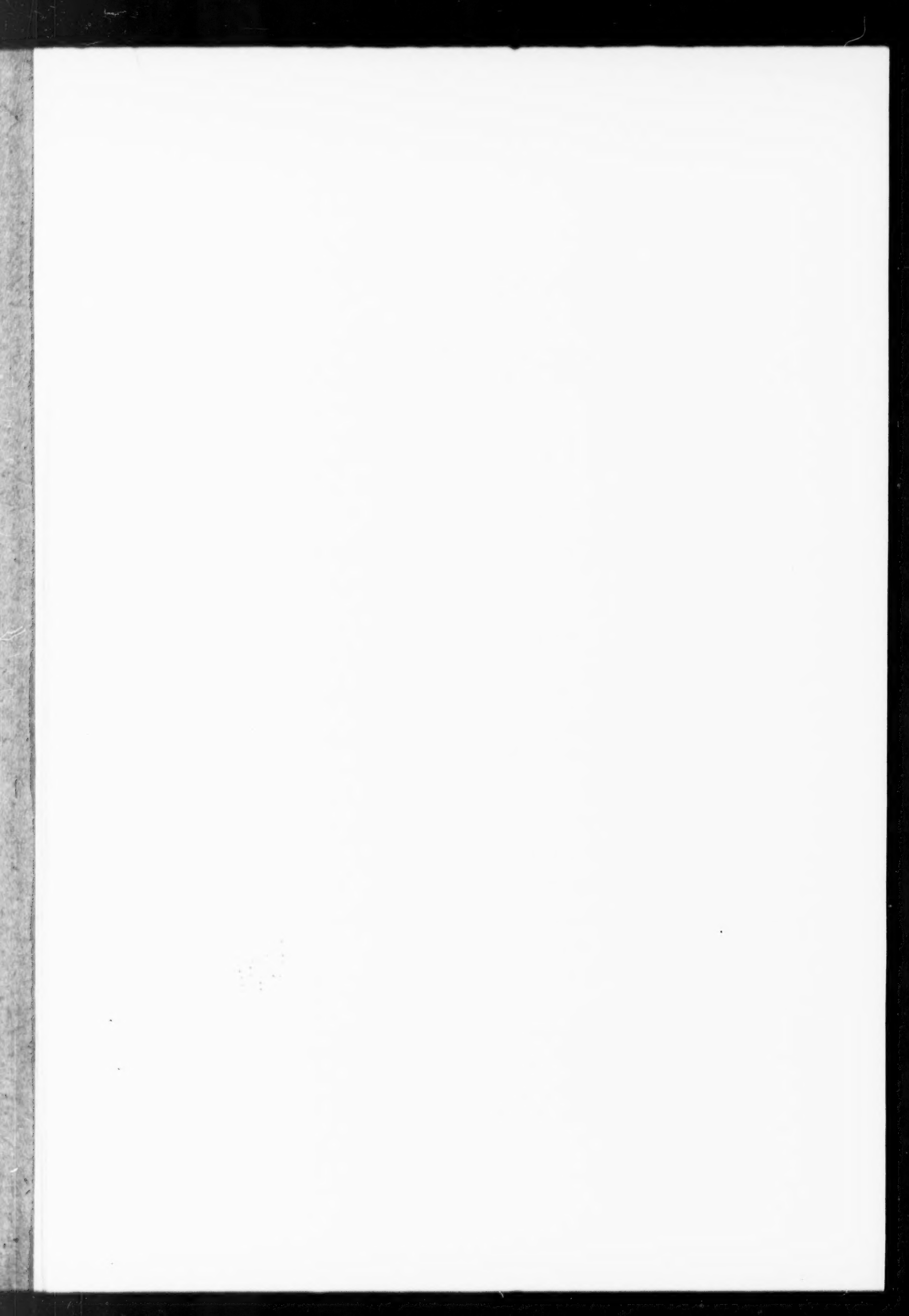
*With Frontispiece, eighteen plates, one hundred and thirty-one  
figures in text, and nine charts*



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*A. Ralliet*

PROFESSOR A. RALLIET

Volume XVI

March 31, 1922

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Edited by

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- (1) Two courses for the Diploma in Tropical Medicine, each of three months' duration, commencing about the 15th September and the 7th January. The D.T.M. examinations are held in December and April.
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# ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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Illustrations for text figures or charts should be drawn clearly and firmly in Indian ink, if possible on Bristol board. N.B.—*Blue or other coloured* ruling in squares or lines cannot be reproduced.

All lettering, names or legends on text-figures, charts or maps should be printed *sufficiently large* to allow of *clear legibility* on reduction if necessary.

Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—‘According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.’ The references should be collected in alphabetical order of authors’ surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). ‘The spleen in malaria.’ *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). ‘Enlargement of the spleen in malaria.’ *Journal of Pathometry*, Vol. I, pp. 1-20.

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# UNDULANT FEVER IN THE GOAT IN MALTA

BY

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GOVERNMENT ANALYST, PUBLIC HEALTH DEPARTMENT, MALTA

*(Received for publication 17 October, 1921)*

## PLATES I AND II

Undulant fever, which, notwithstanding the decisions of International Boards, is still by many called Mediterranean fever or Malta fever, is, as heretofore, a disease which causes much suffering and anxiety.

Since the year 1905, when it was demonstrated that the infection was caused by the drinking of milk, more especially that of goats, the Public Health Department of Malta has been endeavouring not only to minimise the occurrence of the disease, but to find a way to stamp it out. For this purpose it has been instructing the public as to the prophylactic measures it should follow, and at the same time keeping watch over the milch animals on the Islands.

In the ten years (1894-1903) preceding the appointment in 1904 of the Mediterranean Fever Commission, the average number of cases in the Maltese Islands was Malta 3.2, Gozo 1.9 per 1,000 of estimated mean population per annum.

During the years 1901, 1902 and 1903, among the ships of the British Mediterranean Squadron constantly at Malta, with an average crew of 8,230, there was an average of 28.55 cases per 1,000; whereas from 1897 to 1903, amongst the British Garrison on the Islands, there was an average of 25.6 cases per 1,000 per annum.

The apparently greater rate of infection among the Services, as compared with that of the civil population, may be explained by the fact that in the Services every case of illness comes under the notice of the Medical authorities, whereas in the civil population slight cases pass unnoticed, and other, possibly numerous cases, are either incorrectly reported or are not reported at all. This happened more especially before 1904, when, owing to insufficient knowledge, the fever was incorrectly diagnosed, and sufficient importance was not given to it.



When it was found out that the fever resulted from the drinking of goat's milk, and that the micro-organism causing the infection could be destroyed at a temperature below  $100^{\circ}\text{C}$ ., the Maltese Sanitary Authorities at once made it known that the heating to boiling point of fresh milk would free it from infection.

The Naval and Military Authorities were also prompt to take action. Thus in June, 1906, goat's milk was banished from the dietary of the Garrison (*A.M.D. Report* for 1906, Vol. XLVIII, p. 78), while the C.-in-C.'s General Order to the Fleet, dated 4th August, 1906, prohibited the use of unboiled milk (*Navy (Health) Statistical Report* for 1906, p. 119). By these means a disease, the etiology of which had not only baffled the skill of medical men for about a century, but had also affected the efficiency of the Mediterranean Fleet and the Malta Garrison, was arrested; probably a unique case in medical history. The use of fresh milk had no sooner been tabooed in the Services than the number of cases of undulant fever dropped as if by magic, and both the Navy and the Army on this station have since been almost entirely free from it. English people, whose duty compelled them to reside in Malta for some time, obtained a like relief by adopting the simple precaution of boiling the fresh milk or abstaining from it.

Before 1904, few of the employees of the Electric Telegraph Company, the dockyard, etc., or their families, escaped infection; but it is now an exception for a foreign resident, who takes the necessary precautions, to fall a victim to undulant fever. The bulk of the Maltese population, who thought they knew better, have not heeded the caution repeatedly given out by the Sanitary Office as to the danger of using unboiled milk, with the result that there has been hardly any decrease in the number of cases of undulant fever.

### THE GOATS

The Maltese goat is the hardiest, the tamest, the best milking goat in existence. It bears a resemblance to the Theban or Egyptian goat, from which it probably originated. Like the Theban goat, it is generally beardless and frequently hornless, has spreading and slightly pendulous ears, though shorter and narrower, has a convex profile though not so marked as in the Theban goat,

has very often a pair of lappets on the throat, and like it is often of a reddish colour, but it has larger hair and the udders are very large, in relation to its remarkable milking qualities. Maltese goats milking at the rate of  $5\frac{1}{2}$  litres (about  $9\frac{1}{2}$  pints) in twenty-four hours are not uncommon. White haired goats were formerly preferred by goatmen, but it was found that they are less hardy than the reddish or black-haired ones.' (Dr. J. Borg, in 'Malta and Gibraltar,' compiled by Allister Macmillan, p. 237, London, 1915.)

From time immemorial, goats in Malta have been milked at people's doors, and it is impossible by legislation to compel householders to boil their milk, but it has been made unlawful for hotels, restaurants, coffee-houses, etc., to serve other than boiled milk. Such a measure should have helped to educate the people in the matter, but the general public has yet to be convinced that an apparently normal beverage drawn straight from the familiar goat can be productive of a deadly fever.

One must also bear in mind that the conclusions of the Commission have not remained unchallenged; apologists have come forward offering negative evidence in defence of the offending goat. Others, who could see only the financial side of the question, have pleaded the cause of the poor milkman.

The Sanitary Authorities, seeing how their efforts were being thwarted, devised other means to protect the sceptical public. Periodical inspection of goats by trained sanitary officers was instituted, and samples of milk, or blood, were taken. The Widal and the Zammit\* tests are applied to the blood or milk respectively on the day of their collection. If the goat is found to react it is sent to the Lazaretto under escort, and is there examined by a veterinary surgeon, who assesses its value as a dry goat. The

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\* *Widal reaction.* Dilutions of the serum of 1 in 80 and 1 in 100 are made with salt solution. The emulsion of *M. melitensis* consists of one to which formalin has been added. One drop of the emulsion and 1 drop of the diluted serum are mixed. The mixture is placed on one of the  $\frac{3}{4}$ -inch spaces ruled with a diamond on a glass slide about a foot long. The slide is rocked to and fro for about a minute and placed in a wet chamber at room temperature. Results are read after about two hours, but the reaction is usually obtained, in a positive case, after one minute's rocking of the slide. Naked-eye appearance is sufficient, though a hand lens is sometimes used. In this way about 100 specimens of blood can be examined in a day.

*Zammit reaction for milk.* Loopfuls of diluted milk and emulsion of culture are mixed on a slide so as to give resulting dilutions of 1 in 20 or 1 in 40. The mixture is drawn into a capillary tube, which is sealed at both ends and stood on end in sand. The result is read next day, though the reaction is sufficiently clear in a couple of hours, the naked-eye appearance of the precipitate being quite characteristic. In doubtful cases the previous test is applied.

owner has the right of appeal on the question of value. The goat is slaughtered at the Lazaretto. The average annual amount paid as compensation to owners for the destruction of infected goats is about £500. In the estimates for 1921 the sum of £700 is allocated. The average cost of an infected goat in pre-war time was 20s.

It was primarily intended to inspect the twenty thousand odd goats spread over the two islands twice yearly; but the limited special staff available could not cope with the work, and the inspection of goats, sheep and cows is consequently restricted. As the inspections are made periodically only, animals that become infected in the interval remain undetected for some time, and consequently a number of infected goats always exist; hence it is not surprising that undulant fever is still prevalent among the civil population.

The systematic, but limited purification of the herds effectually reduced the disease among the animals, and although the frequent examination of every milch animal, with the consequent slaughter of those found infected, is costly, it should be made continuous if the fever is to be eliminated. A much larger staff should be organised; that available has been unable to inspect yearly more than about six thousand, out of about twenty thousand goats.

According to the reports of the Public Health Department, the number of goats examined and the rate of infection found were as follows:—

TABLE I  
Showing the rate of infection with *M. melitensis* of goats in Malta

Year	No. of goats examined	No. infected	Percentage
1907-08 ... ..	1,203	170	14'1
1908-09 ... ..	1,099	32	2'9
1909-10 ... ..	9,924	461	4'6
1910-11 ... ..	13,372	402	3'0
1911-12 ... ..	13,756	386	2'8
1912-13 ... ..	11,453	414	3'6
1913-14 ... ..	6,896	381	5'9
1914-15 ... ..	4,965	385	7'7
1915-16 ... ..	6,630	598	9'0
1916-17 ... ..	7,768	536	6'9
1917-18 ... ..	5,921	287	4'8
1918-19 ... ..	4,613	187	4'0
1919-20 ... ..	5,690	341	5'9



The infection rate is consequently about 5 per cent., a dangerous percentage, for a single goat may infect hundreds of persons during its milking activity.

Sheep are less liable to infection than goats, probably owing to the smaller size of their udders with a consequent smaller chance of abrasion, but there are no reliable statistics as to the number of sheep infected.

In the light of modern treatment of infective diseases, prophylactic inoculations with a *melitensis* vaccine have been suggested on various occasions. In 1906, Dr. Eyre, one of the members of the Mediterranean Fever Commission, used a vaccine in fifty-one cases; of these, twenty-two received one injection and twenty-nine received two injections of 200-400 million cocci. Two of the cases vaccinated contracted the disease.

Professor M. H. Vincent (1918), of Paris, carried out vaccination experiments on goats and published his results in a paper, in which he claimed to have solved the problem of the *melitensis* infection.

The Maltese Government, wishing to utilize Professor Vincent's vaccine, asked the writer to report on the matter. It was agreed, therefore, to repeat Vincent's experiments as described by him, on a number of local goats. The writer, who was no longer in a position to conduct the experiments himself, had the honour to be entrusted with their supervision.

The Technical staff of the Public Health Department carried out the experiments in a most conscientious manner. They all had long experience, both with the micro-organism and with infected goats, and followed Vincent's directions in all particulars. A full report of the work will eventually be published, so that I will only mention the broad conclusion arrived at, that is, that the bright hopes built on the French savant's paper have been dashed to the ground. The immunisation of the vaccinated animals did not occur, and a minimal dose of virulent culture of *M. melitensis* infected both the experimental animals and the controls.

The question remains therefore, *in statu quo*, and either another vaccine will have to be devised or vigorous direct action be taken to free the island from the fever.

At this point, one cannot allow to go unchallenged an assertion that Professor Vincent made in the above-mentioned paper to the

effect 'that an infected goat recovers spontaneously after a period of time more or less long.' This is a bold assertion, which is intimately connected with the whole prophylactic question of the fever. I do not believe that Professor Vincent is justified in making such an assertion, which is contrary to our experience.

The writer who has a very long experience of goats, both normal and infected with *M. melitensis*, has never known an infected goat to recover. The animal may feed well and look bright, its blood may completely lose its agglutinating power, but a careful post-mortem examination shows, as a rule, the micrococcus lurking in one or another of the glands.

After kidding, a goat, that has for about two years appeared healthy and free from an infection it had previously contracted, yields a milk teeming with *melitensis*.

It is satisfactory to read in the Chief Government Medical Officer's Report for 1918-19, that 'the notified number of cases (three hundred and sixty-three) points again this year to diminished incidence of the disease, with deaths—sixteen—representing a *case mortality* of only 4·4 per cent. This is the smallest on record, and is equal only to a death rate of 7·1 per 100,000.' In the report of 1919-20, however, the disease shows a slight recrudescence (six hundred and nineteen cases with a case mortality of 5·1 per cent.), which reduces the hope for a progressive amelioration.

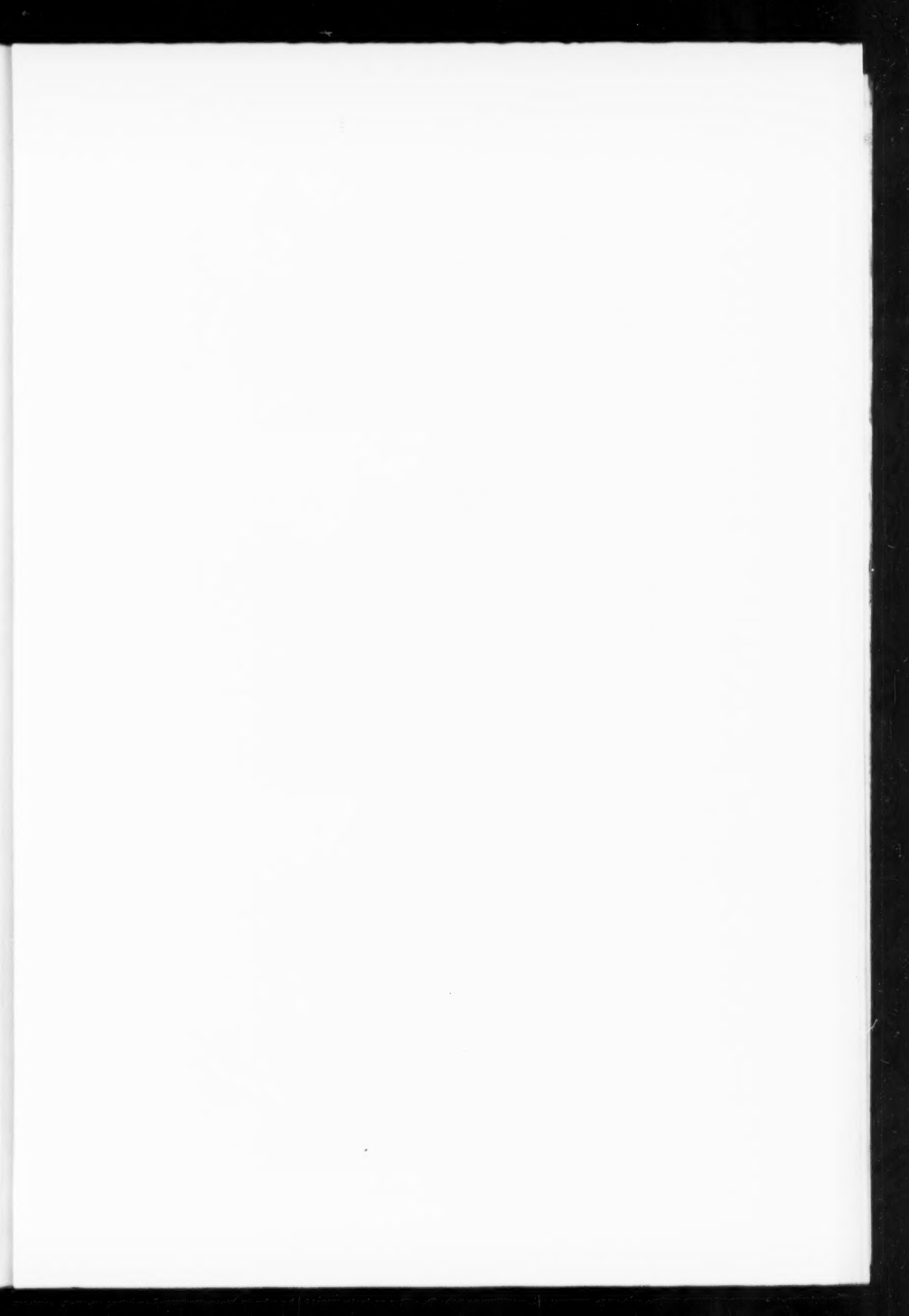
The civil population, too, can protect itself, by using no milk that is not previously boiled. Were this simple procedure to be strictly followed, the fever would disappear from the civil population as it did from the Navy and Army. As, however, most of the people remain obdurate, or careless, it is our duty to eradicate the disease by vigorously acting against the main cause: by destroying every animal found to be infected.

Goats are not susceptible to a cure, and even were a cure possible, their treatment would, in the happiest event, be long and costly; more costly than the animal itself.

An adequate inspection staff should be provided, and no expense spared. Were this done, I am confident that the fever would disappear from the island in a short time.

#### REFERENCE

- VINCENT, M. H. (1918). Sur la prophylaxie de la Fièvre de Malte par l'immunisation active des animaux vecteurs du germe. *Comptes Rendus de l'Académie de Science*, Feb.



## EXPLANATION OF PLATE I

- Fig. 1. Maltese Goat, presented to the Museum of the Liverpool School of Tropical Medicine by Prof. T. Zammit.  
Photo by Miss M. Brown.
- Fig. 2. Group of goats, Malta.
- Fig. 3. Maltese Goat, presented to the Museum of the Liverpool School of Tropical Medicine by Prof. T. Zammit.  
Photo by Miss M. Brown.



FIG. 1



FIG. 2



FIG. 3







EXPLANATION OF PLATE II

- Fig. 1. A milch goat, Malta.  
Fig. 2. Group of goats, Malta.  
Fig. 3. A milch goat, Malta.

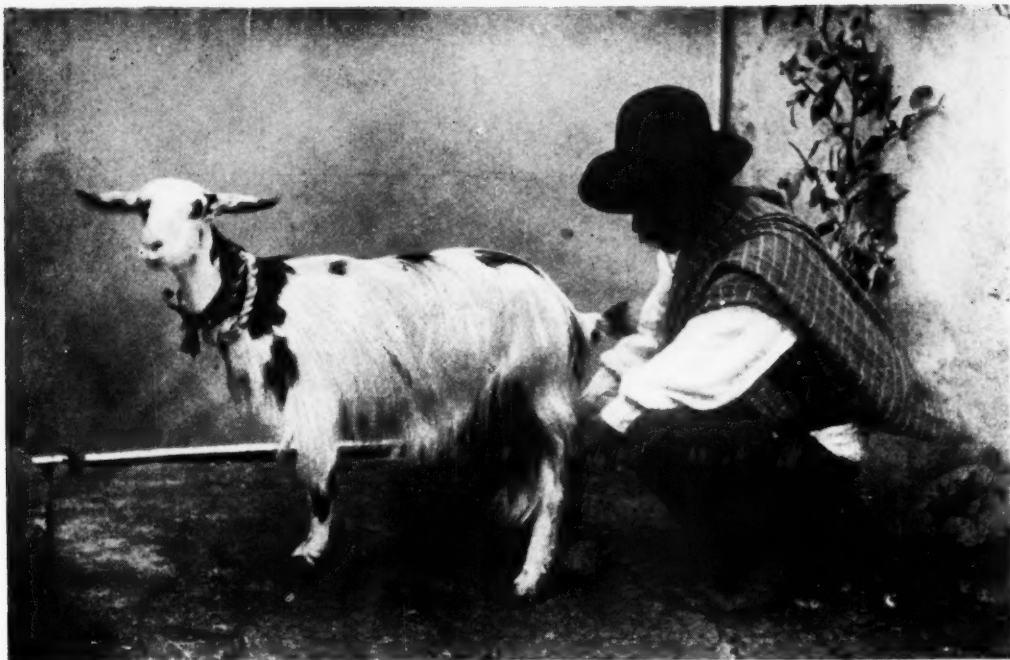


FIG. 1

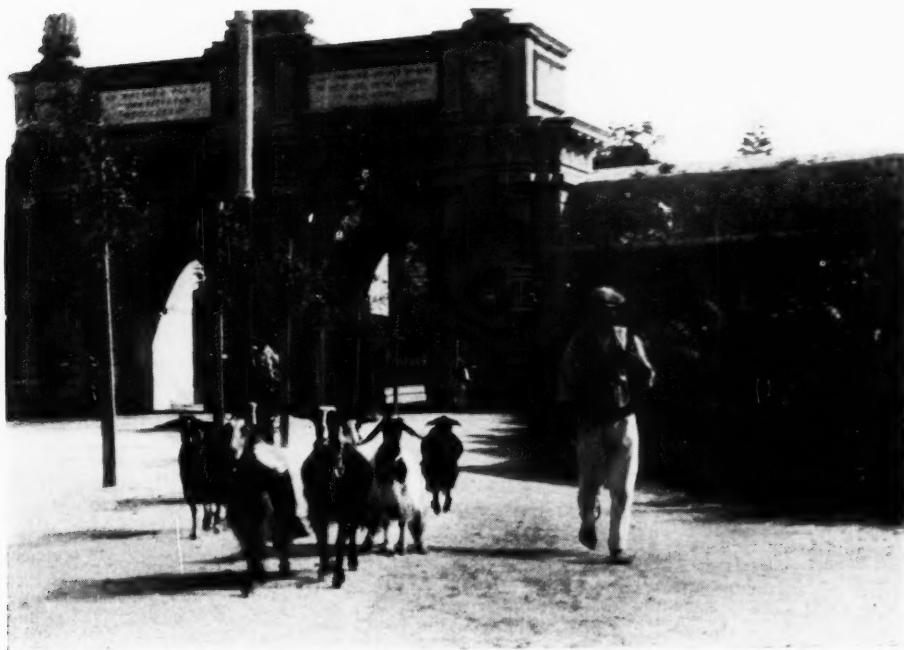


FIG. 2



FIG. 3



# UNDULANT FEVER IN THE NAVAL, MILITARY AND CIVILIAN POPULATIONS OF MALTA

BY

J. W. W. STEPHENS

*(Received for publication 10 February, 1922)*

In the previous paper Zammit has considered the prevalence of undulant fever\* in the goat in Malta. As complementary to that paper I thought it would be of interest to present afresh the data as to the prevalence of the fever in the Mediterranean Squadron, Army (Malta Garrison), and civilian population of Malta respectively previous to 1906, and from that time onwards so far as data are available. 1906 is the critical year in regard to the relationship of undulant fever and goats' milk, for it was mainly in the latter half of that year that orders affecting the use of goats' milk came into force.

I have prefaced each section of the paper by some remarks, with the object of elucidating the meaning of the figures presented; for it is difficult, in the case of many, if not all, vital statistics, to ascertain whether they really represent what they are supposed to do. In the present connection it is essential to know whether a case 'returned' as undulant fever is that fever or not. Undulant fever is among the select class of infections that can be diagnosed with certainty. It is probable not only that certainty has not been attained in many cases, but that the approach thereto is a variable one.

## NAVY

### MEDITERRANEAN SQUADRON

#### I. NOMENCLATURE:

The following terminology is used in the 'Statistical Reports of the Health of the Navy':—

*Other Continued Fevers* is used from 1900-1906 and signifies fevers other than enteric and Mediterranean, influenza appearing under its own heading.

*Pyrexia* replaces 'Other Continued Fevers' in the tables from 1907-1914.

\* In the 'Nomenclature of Diseases,' Royal College of Physicians, London, 1897, 3rd Edition, appears the entry, *Mediterranean Fever*, synonym *Malta Fever*.

In 1918, 5th Edition, the entry, *Mediterranean Fever*, synonym *Undulant Fever* is used.

*Simple Continued Fever* is also used synonymously with *Pyrexia* in the text in 1909.

*Sand-fly Fever*. In the report for 1910, p. 50, it is stated that of the 98 cases of 'Pyrexia' in that year 58 were 'Sand-fly Fever.'

*Cerebro-spinal Fever* has a separate heading in 1912.

*Mediterranean Fever* is used in the tables from 1900-1906 and from 1910-1914.

*Malta Fever* is used in the tables and text from 1907-1909.

## 2. DIAGNOSIS :

1900. 'Agglutination adopted as a routine practice in the Army and shortly after in the Navy.'

(*Report of the Commission on Mediterranean Fever*, Pt. II, 1905, p. 12.)

## 3. GOATS' MILK :

### (a) Naval Hospital.

1906—April 9. 'More stringent measures taken at the Royal Naval Hospital for the sterilisation of goats' milk.'

1906—July 23. 'Preserved milk substituted for goats' milk.'

(*Statistical Report of the Health of the Navy*, 1906, p. 120.)

### (b) The Fleet.

1906—May 23. 'The Commander-in-Chief promulgated a general memorandum to the effect that as a guarantee of sterilisation the ortol and peroxide of hydrogen test should be used in all ships.'

1906—Aug. 4. 'The Commander-in-Chief repeated the order that all milk obtained in Malta was to be boiled. It was then to be tested by the ortol test.'

(*Commission Report*, Pt. VII, 1907, pp. 72 and 73.)

## 4. ESTIMATED STRENGTH :

The figures for the *average strength* refer to the *Mediterranean Squadron* and not simply to Malta, so that the rate per 1,000 is not comparable with that of the garrison or civilian population of Malta. These figures are *corrected for time*, i.e., if 1,000 men have been in the Mediterranean Squadron for six months and 500 for one year, the average strength per annum is 1,000 : or again, if 365 men have been in the Squadron for one day and one man for 365 days, the average strength per annum is 2.

TABLE I

Showing prevalence of Undulant and certain other fevers in the Mediterranean Squadron, 1900-1914

Year	Average strength corrected for time	Cases		Rate per 1000 per annum	
		Mediterranean fever	Other continued fevers	Mediterranean fever	Other continued fevers
1900 ... ..	14250	317	351	22.2	24.6
1901 ... ..	14070	252	323	17.9	22.9
1902 ... ..	18470	354	433	19.1	23.4
1903 ... ..	18410	339	287	18.4	15.5
1904 ... ..	19590	333	401	17.0	20.4
1905 ... ..	14360	270	174	18.8	12.1
1906 ... ..	12130	145	99	11.9	8.1
			Pyrexia		Pyrexia
1907 ... ..	10530	14	110	1.3	10.4
1908 ... ..	9780	6	119	0.6	12.1
1909 ... ..	9920	11	69	1.1	6.9
1910 ... ..	9850	3	98	0.3	9.9
1911 ... ..	9770	5	144	0.5	14.7
1912 ... ..	7870	3	49	0.3	6.2
1913 ... ..	7580	2	38	0.2	5.0
1914 ... ..	10220	6	34	0.5	3.3

## ARMY

### MALTA GARRISON

#### I. NOMENCLATURE :

The following terminology is used in the Army Medical Department Reports :—

*Other Continued Fevers* is used in the statistical tables for the years 1897-1907.

*Simple Continued Fever* is used as one sub-division of ' Other Continued Fevers ' in the text from 1897-1907, the other sub-division being Mediterranean fever from 1897-1903, and Malta fever from 1904-1907.



*Pyrexia of Uncertain Origin* (P.U.O.) replaces 'Simple Continued Fever' in the statistical tables and in the text for the years 1908-1914.

*Sand-fly Fever* appears in the tables and text for 1910-1914.

*Mediterranean Fever* is used in the text from 1897-1903 and also from 1910-1914.

*Malta Fever* is used in the tables and text from 1904-1909.

## 2. DIAGNOSIS :

1900. 'Agglutination adopted as a routine practice in the Army and shortly after in the Navy.'

(*Commission Report*, Pt. II, 1905, p. 12.)

Major-General Sir William Leishman, K.C.B., F.R.S., has informed me that the diagnosis of undulant fever is always based on the agglutination reaction.

## 3. GOATS' MILK :

1905. It is stated that in 1905 'orders were issued by commanding officers that all goats' milk for the use of the men in barracks was to be boiled.'

(*Commission Report*, Pt. VII, p. 168.)

1905—September. The attention of officers commanding was called to the fact that 'in some corps goats' milk had not been boiled before use' (p. 169).

1906—May 16. The ortol test for detecting unboiled milk was in use and 'during the next three weeks neglect of boiling goats' milk was detected on six separate occasions' (p. 170).

1906—May 12. 'Orders were issued for the discontinuance of the use of goats' milk in the military hospitals as a tentative measure, and for its replacement by condensed milk.'

'This change came into operation in the various hospitals between May 18 and 22, and at the same time the use of goats' milk by the various detachments of the Royal Army Medical Corps also ceased' (p. 172).

1906—June. 'By the end of the first week in June all the units of the garrison were using condensed milk, with the single exception of the 1st Battalion Rifle Brigade, which continued to use goats' milk up to October' (p. 173).



TABLE II

Showing prevalence of Undulant and certain other fevers in the Malta Garrison, 1897-1914

Year	Average strength	Cases			Rate per 1000 per annum Mediterranean fever
		Mediterranean fever	Simple continued fever		
1897 ... ..	8023	279	1275		34.7
1898 ... ..	7390	199	1510		27.1
1899 ... ..	7425	275	1107		37.0
1900 ... ..	8140	158	1158		19.4
1901 ... ..	8136	253	1205		31.1
1902 ... ..	8758	155	1029		17.7
1903 ... ..	8903	404	786		45.4
Other continued fevers					
1904 ... ..	9102	320	1350		35.1
1905 ... ..	8294	643	1199		77.5
1906 ... ..	6661	161	508		24.1
1907 ... ..	5700	11	323		1.9
Pyrexia of uncertain origin					
1908 ... ..	6030	5	303		0.82
1909 ... ..	6392	1	285		0.15
Sand fly fever					
1910 ... ..	6769	0	26	124	0.0
1911 ... ..	6686	0	14	125	0.0
1912 ... ..	6593	3	5	104	0.45
1913 ... ..	6336	3	25	72	0.47
1914 (7 months only)	3487	1	0	51	0.28

## CIVILIAN POPULATION OF MALTA

### I. NOMENCLATURE :

' All fevers lasting more than a week are notifiable by law.'

' Mediterranean fever is generally notified under the name of remittent fever.'

(*Commission Reports*, Pt. II, 1905, p. 15.)

It appears from the above Report that in the civil official notification returns, cases notified under the name ' continuous fever ' are included in the Annual Public Health Reports under heading ' Mediterranean fever.' We find the following terminology employed in the Public Health Reports :

*Remittent Fever* is used (for Mediterranean fever) in the Reports for 1897 and 1902-03.

*Continued Fever*, in addition to remittent fever, or Mediterranean fever, is used in the Reports for 1897, 1906-07, and (apparently synonymously with *febricula*) 1907-08, and then disappears.

*Febricula*, in addition to remittent, or Mediterranean, fever, is used in the Reports from 1898 to 1906-07 and then disappears. The number of cases for the five years, 1902-03 to 1906-07, was 66, 35, 26, 22, and 42 respectively.

*Undulant Fever* (for Mediterranean fever) first appears in the Report for 1912-13.

The terms ' Simple continued fever,' ' Pyrexia of uncertain origin,' and ' Sand-fly fever ' do not appear in any of the reports.

### 2. DIAGNOSIS :

That the agglutination test is in use for purposes of diagnosis appears from the Public Health Reports, for in the Report for 1912-13, p. 30, it is stated that ' 636 samples of blood were submitted by private medical practitioners for the agglutination test of cases of fever.'

### 3. GOATS' MILK :

1907-08. The use of boiled goats' milk adopted in the Central General Hospital.

(*Annual Report, Public Health Department*, 1908-09, p. 5.)

1909—June. Regulations were issued apparently at this time requiring that all milk sold in shops, restaurants, etc., be boiled, but it appears from the Public Health Reports (1911-12, p. 43) that ' it is very seldom that the law is complied with.'

TABLE III

Table showing prevalence of Undulant fever and Febricula in the civilian population of Malta, 1902-03 to 1919-20.

Year			Population	Cases		Rate per 1000 per annum Undulant fever
				Undulant fever	Febricula	
1902-3	...	...	193,315	589	66	3.0
1903-4	...	...	197,070	573	35	2.9
1904-5	...	...	202,134	663	26	3.3
1905-6	...	...	205,059	822	22	4.0
1906-7	...	...	206,689	714	42	3.4
1907-8	...	...	209,974	501	6*	2.7
1908-9	...	...	212,888	463	...	2.1
1909-10	...	...	215,879	463	...	2.1
1910-11	...	...	213,395	297	...	1.7
1911-12	...	...	215,332	275	...	1.2
1912-13	...	...	216,617	370	...	1.7
1913-14	...	...	216,879	338	...	1.5
1914-15	...	...	218,542	321	...	1.4
1915-16	...	...	220,968	473	...	2.1
1916-17	...	...	223,741	495	...	2.2
1917-18	...	...	224,326	429	...	1.8
1918-19	...	...	224,655	363	...	1.6
1919-20	...	...	224,859	619	...	2.7

\* 'Continued fever.'

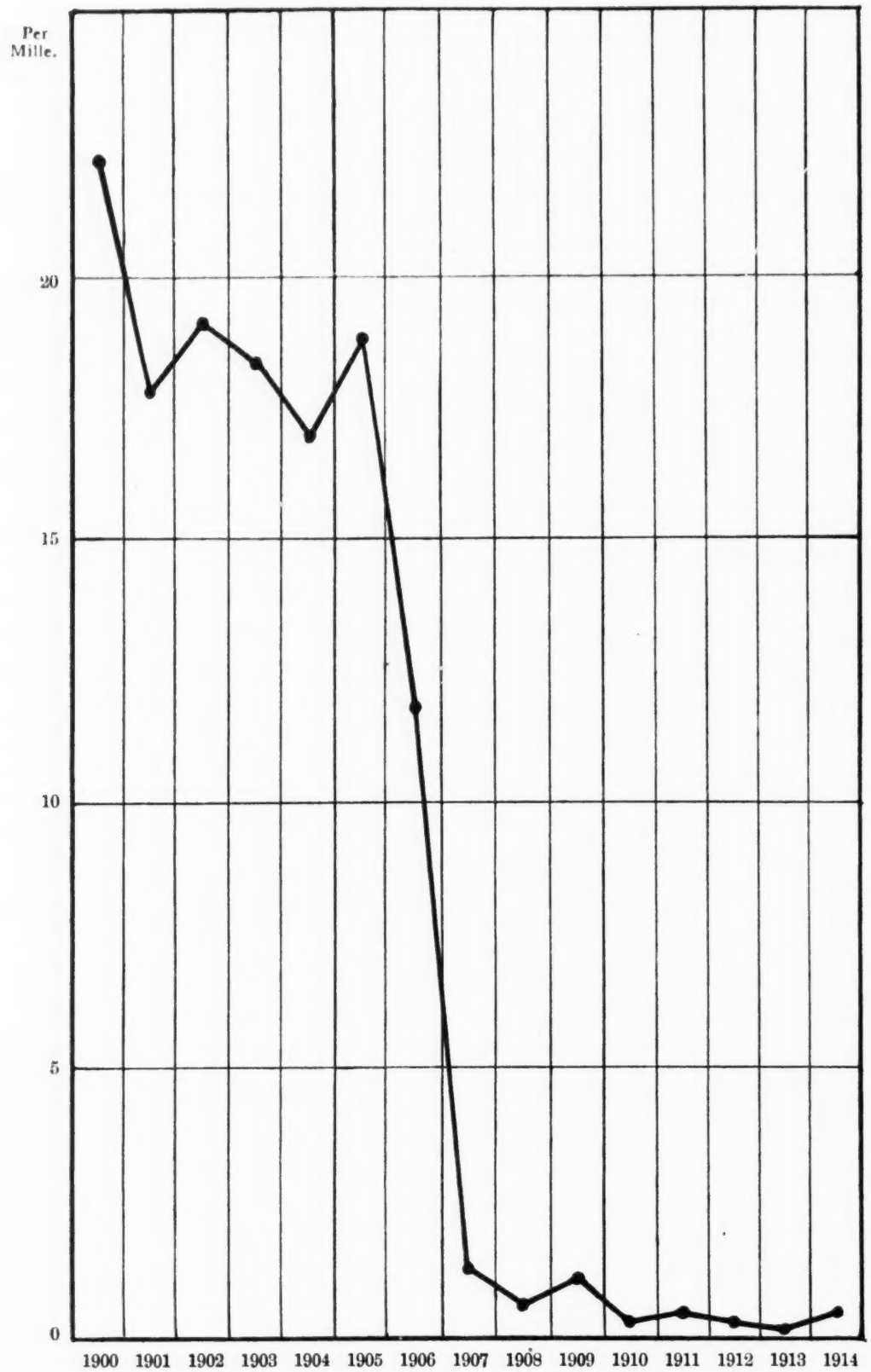


CHART I. Showing incidence of Undulant Fever in the Mediterranean Squadron.

N.B.—One division of this scale represents 5 per 1000.

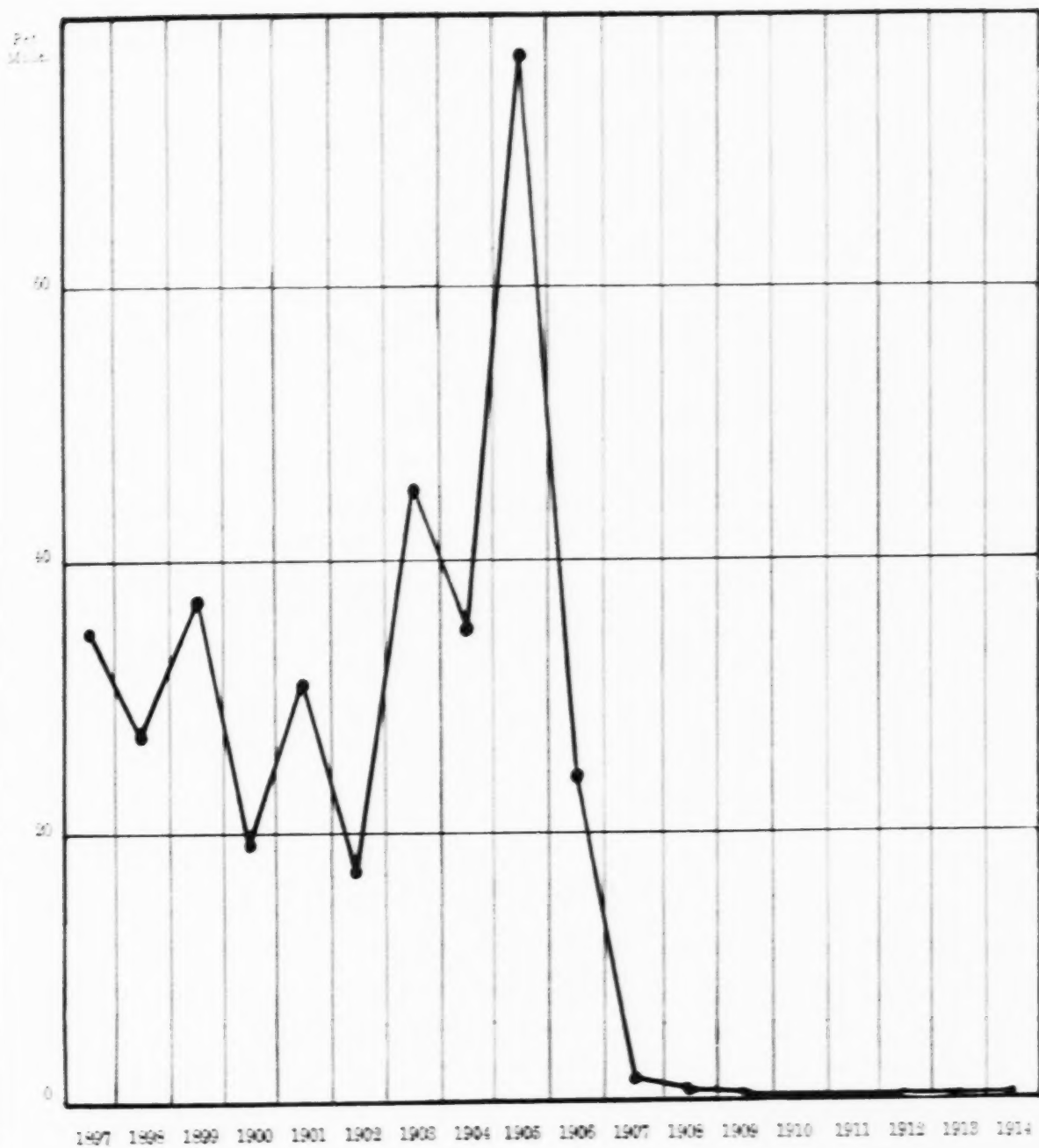


CHART II. Showing incidence of Undulant Fever in the Malta Garrison.

N.B.—One division of this scale represents 20 per 1,000.

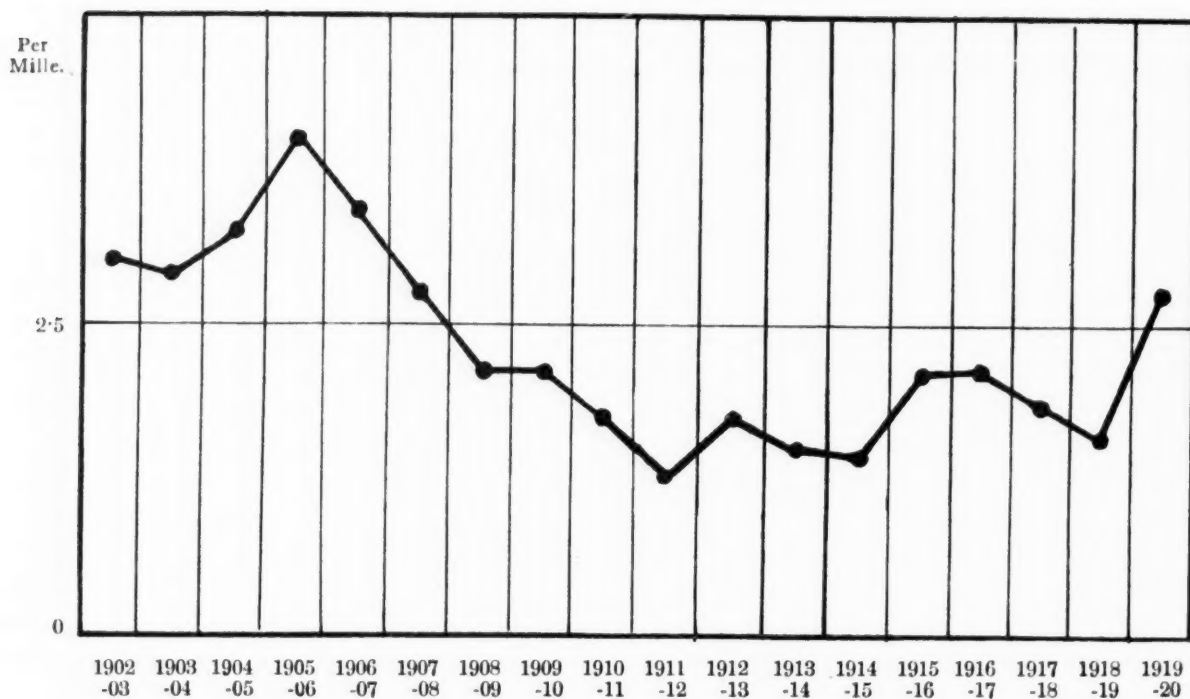


CHART III. Showing incidence of Undulant Fever in the Civil Population of Malta.

N.B.—One division of this scale represents 2.5 per 1,000.

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continued as  
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continued as  
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- Reports of the Commission appointed by the Admiralty, the War Office and the Civil Government of Malta, for the Investigation of Mediterranean Fever, under the supervision of an advisory committee of the Royal Society. Parts I-VII. London, 1905-1907.



# ALASTRIM; OR, KAFFIR MILK POX

BY

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## PLATES III-VII

For some months before my arrival in the Colony an epidemic of an eruptive fever described as Alastrim, or Kaffir Milk Pox, broke out in Kingston, and from Kingston spread to the other parts of the Island. From about May, 1920, to the end of March, 1921, two thousand nine hundred and twelve cases have passed through the Isolation Hospital at Bumper Hall, Kingston, and about six thousand have occurred throughout the Island.

In the following paper I propose to consider:—

- I. The clinical aspect of the disease (p. 21);
- II. Its occurrence in the foetus (p. 29);
- III. Its relation to vaccination (p. 32);
- IV. Its morbid anatomy (p. 34).

### I. CLINICAL ASPECT

*Incubation period.* Owing to the difficulty of getting cases in which exposure occurred only once, and that for a short time, it has been impossible to determine the exact period of incubation; but in those in which I was able to get some definite history of exposure the incubation period varied from ten to about fourteen days.

The evidence on which this conclusion is based is as follows:—

CASE 1. A.H. Not vaccinated. He was in Kingston for three days during July when the epidemic was limited to Kingston only, and returned to his country district, where no cases had hitherto occurred. The symptoms developed 10 days after his return.

CASE 2. Photographer E. Not vaccinated. He attended with me at the Isolation Hospital and took his first set of photographs on July 20th, 1920. During this visit he placed his focussing cloth, before using it, on a chair which had been previously occupied by a patient. Ten days later he again took a photograph, and 3 days after the 2nd photograph he developed symptoms. He was positive

that these were the only two occasions on which he was exposed. Fortunately, his case proved to be mild in character.

CASE 3. Nurse at the Isolation Hospital. Not vaccinated. She complained of headache and pain in the back 14 days after she took up duty. So far as she knows she had not previously been exposed. The disease ran its usual course. This case had been exposed to infection several times before symptoms developed and is only useful as determining the upper limit of the incubation period.

CASE 4. A boy at school in Kingston. He developed symptoms at Annotto Bay 11 days after leaving his school in Kingston. He stated that a number of cases of Alastrim had occurred at his school, but he did not know when he had himself been exposed to infection. He was the first case which occurred in Annotto Bay, and like Case 1, was directly traceable to Kingston.

The remaining cases are not so definite.

CASES 5-7. During their stay at the Isolation Hospital a number of pregnant women suffering from Alastrim gave birth to children. Three of these cases I saw. The infants at birth were free of all signs of the disease. They were breast-fed by their mothers, and the rashes appeared on the 10th, 11th, and 12th days after birth respectively.

Not much weight can be attached to this evidence because intra-uterine infection could not definitely be excluded. I say this because later on in this paper I shall instance such cases in which infants were born with the rash well developed.

Failing more definite evidence, the period of incubation can therefore be placed provisionally at from ten to fourteen days.

*Onset.* The onset of the disease is sudden. There is a rise of temperature accompanied by headache and backache, and occasionally pains in the limbs and vomiting. The rise of temperature was constant.

Of two hundred and two cases of both sexes (one hundred and thirty-three males and sixty-nine females), the incidence of the various symptoms of onset were as follows:—

Headache ...	...	172 cases, or about 85 per cent.
Backache ...	...	111 „ „ „ 54 „
Pain in limbs ...	...	41 „ „ „ 25 „
Vomiting ...	...	32 „ „ „ 16 „

The headache, when present, was generally severe, and often either frontal or vertical.

Of the one hundred and eleven cases in which backache occurred, only forty-five described their pain as severe; the remaining sixty-six described it as moderate. Backache was relatively far more frequent

among the women than among the men; 70 per cent. of the former complained as compared with only 45 per cent. of the latter, and twenty-seven of the forty-five severe cases were among the sixty-nine women. The greater incidence of backache among the women is probably due to the fact that many of them were victims of chronic endometritis, and magnified their usual backache symptoms.

The combination of headache, vomiting and pain in the back occurred in only twenty-one of the two hundred and two patients, and of these only six vomited more than once, and only one more than three times. In the majority of cases the tongue was furred and constipation was present.

*Other manifestations.* The characteristic eruption appeared with about equal frequency on the third or fourth day after the onset of the symptoms. The actual figures are as follows:—

In 21 cases	the rash occurred on the	2nd day.
In 75	„ „ „ „	3rd „
In 70	„ „ „ „	4th „
In 36	„ „ „ „	5th „

Either shortly before or after the appearance of the rash, the temperature falls and the constitutional symptoms disappear. The patient is then quite at ease until maturation begins, when for two or three days there is a great deal of pain from the tension under the skin. In a number of cases there is also secondary fever. No prodromal rashes were seen. Delirium was never observed. The deep depression which occurs at the onset of true smallpox was uniformly absent.

*Menstruation* did not appear in the women unless a period was due, and even then no one complained of more than her usual loss of blood.

*Odour.* There was an absence of odour such as is produced by smallpox. A few cases, however, developed a distinctly putrefactive smell, which was due to decomposing discharges.

*Pain in the throat and dysphagia*, accompanied in some cases by aphonia and enlargement of the glands of the neck, were noted as occurring in a number of cases. These symptoms were due to the presence of the eruption on the fauces, and presumably in the larynx and trachea.

*Sputum.* Three cases had bronchitic signs in the chest, and for a few days coughed up blood-stained sputum.

*Bowels.* In two cases there was profuse diarrhoea at the onset, but the majority were constipated.

*Urine.* In fifty cases whose urines were examined albuminuria was absent, unless due to some other cause, such as urethral or vaginal discharge. Unfortunately, no urines were obtained before the eruption appeared, and in none of the cases was the examination performed more than once. In one case of diabetes, the eruption ran the usual course, but was followed by a large number of boils.

*Eruption.* Patients do not usually come under observation until the rash is well developed; but in two cases which were admitted to the Isolation Hospital in the pre-eruptive stage the rash appeared in the form of small papules, which to the touch were superficially situated: the papules becoming vesicular in about thirty-six hours.

The vesicles are circular in shape, and when fully mature are from 4 to 5 mm. in diameter. The summit is either dome-shaped or flattened, and frequently shows a darkened central area. In the early stages the vesicles, if pricked, yield a clear serum quite free from cells, but polynuclear leucocytes begin to appear in the fluid on the second or third day, and gradually increase in numbers until turbid fluid, or even sometimes thick pus, is formed. At this stage the lesion is very tense, hard and shotty.

In the lighter coloured skins a definite red areola surrounds each pock. Primary umbilication is not often, if ever, seen, but on about the eighth or ninth day a secondary umbilication or flattening takes place, and is due to resorption of fluid.

The eruption is subject to variation, but, broadly speaking, two main types are distinguishable; the one type being finer and more closely set, and the other being larger and more distinct. Sometimes both types are found in the same patient, the vesicles then presenting a very unequal appearance.

The finer eruption has far less tendency to form thick pus, but the general course was similar to that of the larger variety.

A number of confluent and two haemorrhagic cases occurred in this series (altogether four cases of haemorrhagic rash have been brought to my notice, all occurring in women six to seven months pregnant, and all fatal).

*Distribution of the rash.* The rash makes its appearance or, at all events, is first noticed in certain positions. These are in order of frequency, the face, especially the forehead, and the dorsum of the wrist or forearm.

Of the two hundred and two cases, the location of the onset, as noticed by the patient, was as follows:—

Face	...	...	...	...	...	120
Wrist and forearm	...	...	...	...	...	52
Both arm and face	...	...	...	...	...	27
Scrotum	...	...	...	...	...	1
Inner side of knee	...	...	...	...	...	1
Elbow	...	...	...	...	...	1

Although in severer cases, as in Plate III, the whole body may be covered, the rash shows a predilection for certain areas. It especially tends to affect the face, the lower half of the back, and the arm and forearm, especially towards the wrists.

*Scalp.* The rash was present on the scalp in all the cases examined; the lesions, however, were often few in number.

*Mouth.* Pocks were frequently seen on the hard and soft palate, and to a less extent on the pillars of the fauces and the inside of the cheeks. In four cases the fraenum linguae was also affected.

*Larynx.* Hoarseness of voice and sometimes aphonia were present in the majority of the severe cases, and in a fair proportion of the other cases. Laryngoscopy was not possible, but in three of the cases pocks were present in the larynx and trachea—post-mortem.

*Palms and soles.* In all the two hundred and two cases pocks were seen on the palms and soles. In some these were abundant and caused much pain and discomfort. No lesions under the nails were noticed.

*Genitalia,* especially the prepuce, were often affected, and there was in a few cases much swelling and pain and difficulty of micturition.

Plates IV and V show that the rash is present on the area between the knee and the ankle. In true smallpox this area is described as being often free from rash.

The parts on which the distribution of the rash is often comparatively slight are:—



1. The neck.
2. The upper part of the trunk, and the abdomen.
3. The inner side of the thighs.
4. The circumorbital area.

In this latter situation there is frequently no rash at all, even in severe cases, though pocks are often seen on the edge of the lids. No pocks were seen on the conjunctiva.

The effect of irritation appears to be to determine a plentiful outcrop of rash (Plate V).

*The course.* The rash does not appear in crops, but it is often two or three days before the full extent of the eruption is obvious. The order in which it affects the various parts of the body is similar to that of true smallpox. After its appearance it gradually passes through the vesicular stage, already described, until it reaches maturity at about the sixth or seventh day. There is no tense shotty feeling until the rash is nearly matured.

This maturation is accompanied by oedema of the subcutaneous tissues. In the majority of cases this oedema is slight, but in others it is so great as sometimes completely to close the eyes. The oedema appears on the third or fourth day of the rash, reaching its height on the seventh or eighth day, and rapidly disappears (Plate VI).

Resorption of fluid begins to take place on about the eighth day, and convalescence is so rapid in many cases that by the twelfth day nearly all the scabs have fallen off the face. The rash disappears in the same order in which it appears, and in uncomplicated cases all the scabs have fallen at latest by the end of the third week.

In yet other cases the pigmentation is around the scar, the scar itself being achromic. In yet other cases, in fair skins there has been no subsequent pigmentation. In my opinion, the pigmentation is not of much import, in that the normal negro tends to deposit excess of pigment in and around scars. At first I thought that the pigmentation was the result of local treatment, but changed my mind when I saw the same thing in the scars of two infants born alive after intra-uterine alastrim.

*The Temperature.* The onset of the disease is marked by a rise of temperature, which may reach  $104^{\circ}$  or even  $105^{\circ}$ , but in most cases is about  $103^{\circ}$ . This temperature persists with but slight variations for three or four days, then rapidly falls to normal as the rash appears. Sometimes the fall of temperature completely



precedes the appearance of the rash, at other times both take place co-incidentally. The temperature then remains down for four or five days, to rise again as the rash matures. In mild cases there is no

CHART I.—Mild case of Alastrim. No secondary fever. Acute onset.

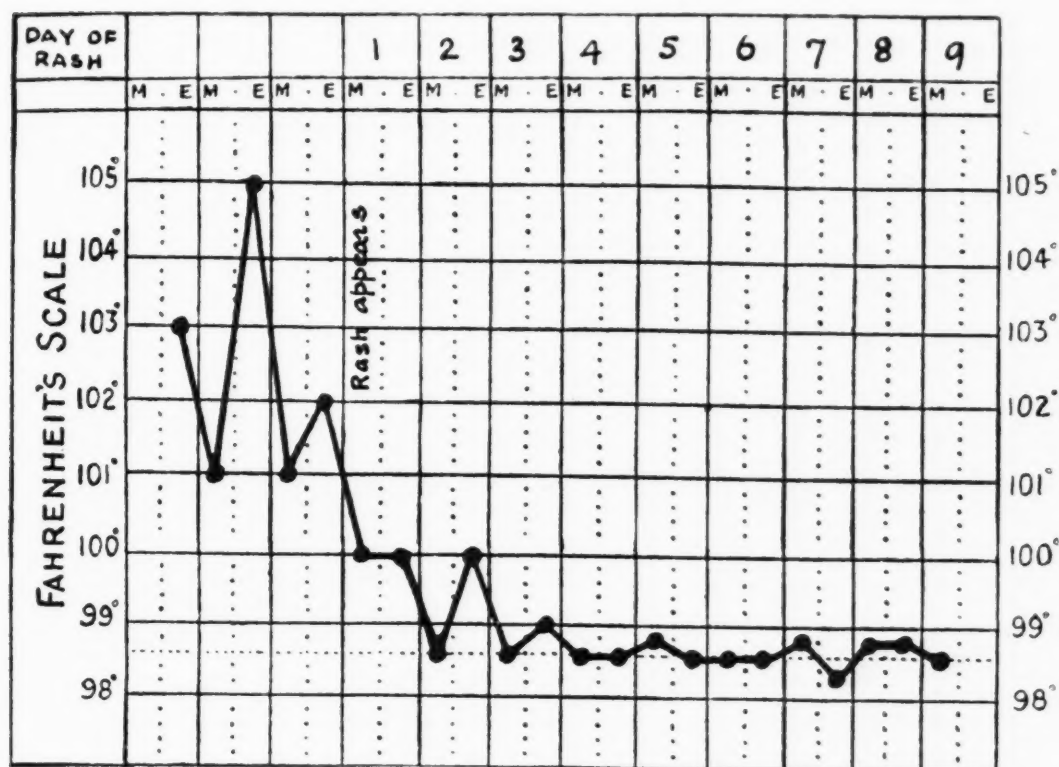
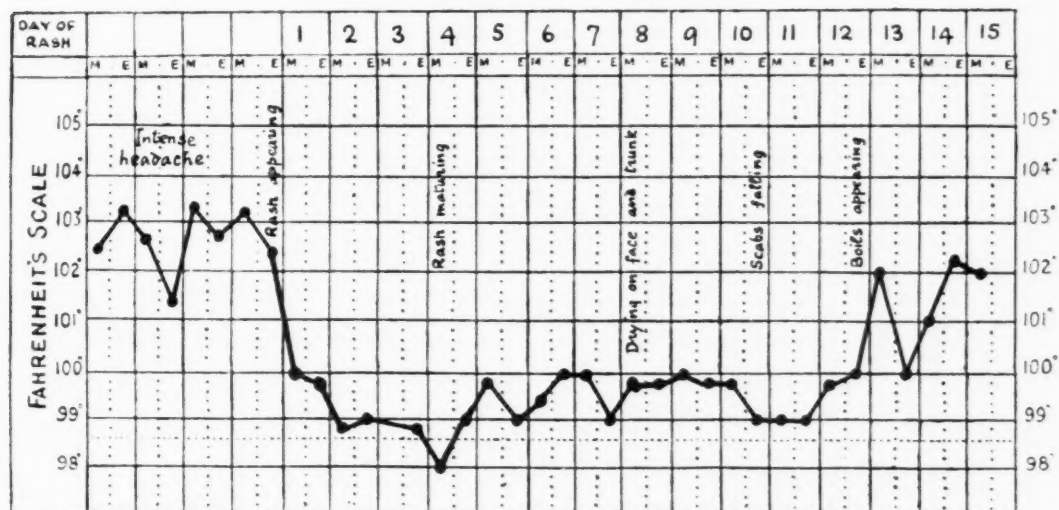


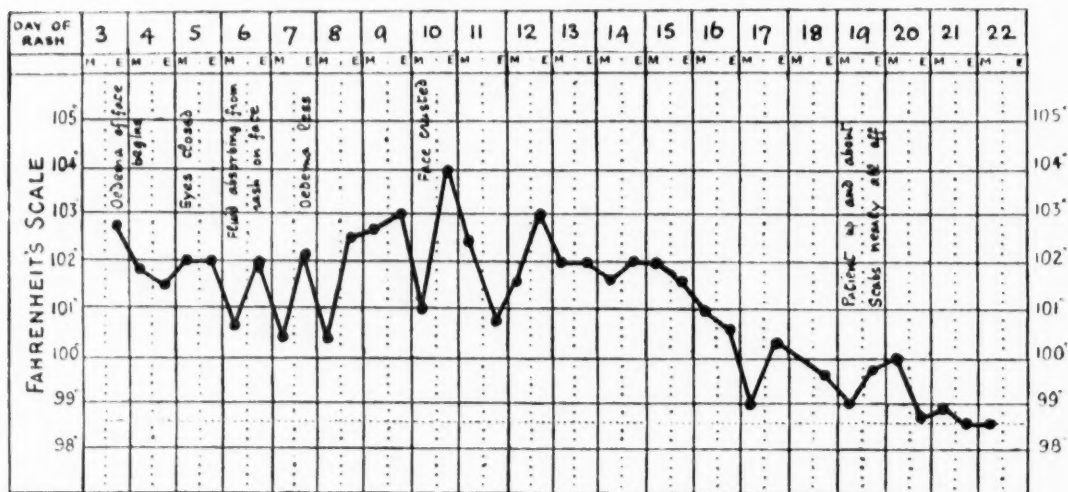
CHART II.—Medium case of Alastrim.



secondary rise of temperature, and in the severe cases the rise appears to be in some way dependent on the extent of the vesicles and the amount of infection with skin organisms. There is only a

slight rise when the pus is practically free from organisms, and a greater rise when there are many. In milder cases secondary fever is absent. A curious point about this secondary fever is that the patient is not conscious that he has a temperature, though his temperature may be as much as  $102^{\circ}$ . After persistence for a few days the temperature returns to normal, and stays there unless complications, such as boils, occur. The secondary fever is as a rule very mild.

CHART III.—Case of Alastrim exhibiting 'Typhoid' type of chart.



There is, however, another type of temperature which has been noted in a few of the very severe cases, and this approximates to the typhoid type, persisting for fourteen or fifteen days before falling to normal.

The temperature of onset is no indication of the severity of the disease, high temperatures being often succeeded by a scanty rash.

#### *Complications and Sequelae.*

*Broncho-pneumonia* is the most serious. It occurred in some of the fatal cases in which much rash was present in mouth and respiratory tract, and was probably due to aspiration of septic material.

*Laryngitis and aphonia* occur in the severer cases, but disappear as the rash disappears.

*Conjunctivitis* of a mild type develops in a number of cases, and is due to infection from the discharges of lesions on the eyelids.

*Impetigo.* Six cases developed impetigo when the rash was disappearing. In severe cases large areas of skin are apt to be

stripped off, leaving raw surfaces which are very painful and troublesome to treat.

*Boils* are the most frequent sequel. They appear at about the fifteenth day after the onset of the rash, and may persist for weeks.

*Eczema* of an intractable character of the external auditory meatus has also been noted.

*Prognosis* is good, except in the newly born and in the haemorrhagic type of rash.

Of two thousand nine hundred and twelve cases which have passed through the Isolation Hospital up to the end of March, 1921, there have been only thirteen deaths, an average of 4.5 per 1,000.

In eight of these cases the condition was as follows:—

Two women who were 6-7 months pregnant with a haemorrhagic rash. One bled profusely from nose and mouth, vagina and bowel; and both post-mortem showed internal haemorrhages.

One man who was 56 years old and died during convalescence.

One man who died after admission, but showed no signs of alastrim.

One man admitted in a dying condition. He had extensive confluent lesions with skin stripping and leaving large raw surfaces. He could hardly breathe. The mouth was very septic, and the smell from putrefying discharges was very offensive.

Three children all within the first month of life and manifesting the disease within the first fortnight of birth.

## II. OCCURRENCE IN THE FOETUS

The virus passes fairly readily through the placenta into the foetal circulation.

Up till February, 1921, of twenty cases admitted, after attacks of alastrim, to the Jubilee Maternity Hospital, eight cases of abortion at about the sixth month have occurred, and in each case the macerated foetus was marked with scars of the disease. Two of the cases I was able fully to investigate, and in these the abortion occurred eight weeks after the onset of the disease in the mother. All the organs were searched for spirochaetes without result, and the Wassermann reaction of the blood of the mothers was negative. The scars in the foetus were slightly depressed.

In addition, two children were born alive with marks of alastrim. The first child was born at full term with marks present as follows:—

Face ... .. 9 marks.

Trunk (front) ... .. 10 „

Trunk (back) ... .. 12 „

A few on each leg and arm.

Two on each sole and one on each palm.

These scars were depressed and surrounded by pigmentation. The mother of this child was alone in the world and developed eclampsia, and died soon after the birth of the child, so that it was impossible to obtain an accurate history. Scars and pigmentation such as occur after alastrim were, however, present on her body.

The second child was born at the seventh month and had no sign of disease on the face, but six spots on the left arm and seven on the right, with three on each leg. These scars were pigmented, as is the case in adults.

The mother's attack occurred eight and a half weeks previous to the birth of the child. The mother's Wassermann reaction was negative.

In none of these cases of foetal alastrim was the disease very severe in the mother, judging by the amount of scarring and pigmentation present.

The remaining ten labours yielded normal, full term children, two of which developed alastrim a day after labour. In addition, two remarkable cases have occurred in which mothers who had been vaccinated, who have never had alastrim, gave birth to children covered with an alastrim rash.

Both cases present a very similar history, save that one mother was vaccinated six weeks and the other four weeks before labour. I give the details of the second case.

'P.C.,' age 23. Sailed from Cuba, January 10th, for Jamaica; was vaccinated on the day of sailing. She landed in Jamaica January 12th, and had fever on 14th and 15th January. So far as she is aware she has never come into contact with any active cases of alastrim. On February 10th (day of examination) the scab had not yet fallen off her vaccination mark and covered an area a little larger than that of a threepenny-bit. She had no signs either in the way of scars or pigmentation of having had alastrim.

The child was born at full term with a pustular eruption (Plate VII), and died five days after birth. Mother and child gave negative Wasserman reactions.

These are cases either of generalised vaccinia occurring in utero



or of alastrim transmitted to the foetus by a mother rendered immune by vaccination. If they are cases of generalised vaccinia they demonstrate that ordinary vaccination can be so transmitted; if they are cases of alastrim, it would appear that just as the diphtheria bacillus grows readily in diphtheria antitoxin so the organism of alastrim can flourish in the blood of one who has, by vaccination, been rendered immune to its toxin, can retain its virulence, pass through the placenta and affect the foetus.

There is finally the possibility that the disease may have existed in the mother, but was so mild as to have been completely overlooked even by herself. If the incubation period be regarded as twelve days, and if the mother were infected on the twelfth day, the day of landing in Jamaica, she ought to have manifested symptoms on January 24th, at a time when, on general principles, she would have been completely protected by vaccination. The child at birth had a rash of at least five days' duration, and if another three days are allowed before the rash appears, must have been manifesting symptoms in utero by the 2nd February. If the child were infected twelve days previous to the manifestations of symptoms, the time relations would be about right. But as against this conclusion, the mother maintains that, apart from slight fever on the fourth and fifth days after vaccination, she was perfectly well. Moreover, I have not seen a mild case of alastrim in which there has not been some malaise. Of the three mildest cases which came under my notice one had four and two had two pocks each, yet in each of these cases the eruption, such as it was, was preceded by fever and malaise. Therefore, pending further evidence, I am of opinion that these cases illustrate the possibility of an immune mother transmitting the disease to her unborn child.

In connection with the question of foetal alastrim, I am impressed by the relatively high frequency with which it occurred among the cases of labour admitted to the Jubilee Hospital. Of twenty cases, ten produced alastrim foetuses, two produced infants developing the disease one day after birth, and only eight produced normal infants.

### III. ITS RELATION TO VACCINATION

The following two tables summarize the facts in two hundred and six adults and eighty children taken at random :—

ADULTS (206)

Cases		Mild	Medium	Severe
		%	%	%
With vaccination scars ... ..	72	47·2	30·5	22·2
Without vaccination scars ... ..	134	28·3	34·3	37·3
Total ... ..	206	35·0	33·0	32·0

CHILDREN (80)

Cases		Mild	Medium	Severe
		%	%	%
With vaccination scars ... ..	26	84·6	15·4	0·0
Without vaccination scars ... ..	54	44·4	25·9	29·6
Total ... ..	80	57·0	23·0	20·0

It will thus be seen that :—

1. There is a tendency to a mild type of case in children, due partly to vaccination and partly to some other factor.

2. No severe case occurred in the vaccinated children of this series, and twenty-two of the twenty-six (84·6 per cent.) vaccinated were mild cases.

Professor MacCallum and myself were continually exposed to infection for hours at a time, but never contracted the disease. We were both vaccinated, he recently and myself five years ago. One of the helpers at the Isolation Hospital was vaccinated by me before taking up duty. I frequently watched her handle the patients and



then either put her unwashed hands to her mouth, or wipe them in a handkerchief which she subsequently used to wipe her face. She never manifested any symptoms.

The Medical Officer, of Health, Kingston, has vaccinated more than five hundred contacts, and he states that no cases of alastrim have occurred amongst them. I have vaccinated twenty contacts, and these were also completely protected.

On the other hand, five cases occurred in vaccinated infants, three in infants two years old, and two in infants four years old.

Goldsmith and Loughnan (1921) give clinical notes of three cases occurring in the vaccinated, three years, one and a half years and one year after successful vaccination.

*Re-vaccination after alastrim.*

Sixty cases were vaccinated by me during convalescence, the time of vaccination varying from the fifteenth day after the onset of the rash up to the twelfth week.

*Of these, fifteen showed no sign of 'take' in two weeks, but twelve of these fifteen were subsequently vaccinated with three 'takes.'* The method used was that of simple incision through the surface layers of the skin. Of the forty-five 'takes' none was typical as compared with normal controls vaccinated with the same batches of lymph.

*Course.* The incision healed over, and no sign of 'take' was visible before the seventh or eighth day, when a few papules were seen in the line of the incision. The papules increased but slowly in size, and by the fourteenth day were raised about 2 mm. above the surface of the arm. No induration or swelling of the arm was noticed, and in only a few cases was there adenitis, temperature, malaise or an areola more extensive than  $\frac{1}{2}$ -c.m. around the lesion, and even in these few cases the symptoms were hardly noticed. On pricking the early vesicles a small quantity of clear fluid was obtained. The lesion was multilocular, and on removing the top of the vesicles in a few cases it was seen that the base was composed of exuberant granulations rising up above the level of the skin, accounting for the fact that sometimes on pricking a small amount of blood came out with the fluid.

The scabs fell off in four weeks or more, and with one exception the resulting scar was not the depressed, pitted scar of typical

vaccinia, but on the contrary slightly raised (hypertrophy) above the surface, subsequently contracting to the level of the surface.

The points of difference between this and typical vaccinia appear to be :—

1. Tardy development and course of lesion.
2. Small size of vesicles and exuberant base and imperfect umbilication.
3. Insignificance of both local and constitutional reaction.
4. Resultant scar.

The evidence of the nature of vaccination after alastrim seem to show that vaccine lymph contains something else beside the factor which protects against alastrim, and it is this something else which gives the reaction after alastrim. The vaccine lymph on examination was found to contain a large amount of *Staphylococcus aureus*, an organism which is present in many specimens of calf lymph, and it may be this organism which causes the reaction, but the lesion produced did not suggest a septic process.

I inoculated four rabbits with the fluid from the vaccine lesion of one of the post alastrim cases, but the results were negative.

My opinion is that alastrim and vaccinia belong to the same group but present slight individual differences, the one disease affording almost complete immunity to the other.

#### IV. MORBID ANATOMY

CASE I. Post-mortem performed 16 hours after death. Death took place on the 12th day of disease. Patient thickly covered with rash, which was confluent in parts and presented inequality of size of vesicles. Small petechial haemorrhage was seen on the sides of the abdomen. Patient was 6 months pregnant. She was admitted bleeding from nose, mouth, uterus. The mouth and throat were filled with a mass of necrotic material, and the entrance to the larynx and the vocal cords was similarly covered with necrotic material. The trachea contained pocks in its entire length. The right lung showed a haemorrhagic condition. There was a broncho-pneumonia with small spots of scattered haemorrhage visible on the cut surface of the lung. The left lung was apparently affected in the same manner, but to a less extent. The heart showed petechial haemorrhages under the epicardium, especially at the root of the aorta, and gross haemorrhage into the muscle substance of the left ventricle. Valves normal. The kidneys presented gross haemorrhage in the medulla and the pelvis was filled with blood. The bladder also showed haemorrhage under the mucous membrane, especially around the outlet. The liver was enlarged, but to the naked eye not obviously abnormal. Spleen not enlarged, firm. Stomach filled with 'coffee grounds' material,

petechiae present under mucous membrane. Intestines: colour dark red, with much haemorrhage and oedema. This condition affected the duodenum and first 3 feet of the jejunum. No lesion seen in the foetus.

CASE 2. Post-mortem six hours after death. Patient well covered with rash, which, on the face, was beginning to crust. Some confluence present on left side of abdomen. Patient 7 months pregnant. The throat was filled with necrotic material as also was the entrance to the larynx. Trachea showed the presence of pocks in its entire extent, and a similar condition obtained in the bronchi. Right lung showed some small haemorrhages and broncho-pneumonia. Left lung apparently normal. No haemorrhage into pleura or pericardium. Heart: A few spots of haemorrhage over the right ventricle under the epicardium. No gross haemorrhage into the substance of the muscle. Valves normal. Liver much enlarged and fatty. Spleen normal. Kidneys normal in size, but pale and fatty. Capsule stripped easily. No gross haemorrhage seen. Bladder normal. Uterus: A large subperitoneal haemorrhage on the right side just below the fallopian tube. Uterus contained a 7 months' foetus, which, on examination, showed a few sub-epicardial haemorrhages. Stomach normal. Intestines: The first 2 feet of the jejunum presented a remarkable condition; they were dark, haemorrhagic, and very oedematous. The whole lumen was in parts a solid mass.

CASE 3. Death on 11th day of rash. Scabbing on face and in upper part of chest. There were areas on the arms, abdomen, and particularly on the legs, in which absorption of the fluid in the vesicles was taking place leaving the vesicles lax. There were also large areas of confluent eruption about the size of half-a-crown on the outer portion of both legs, and on the sides of the abdomen. Some of the fluid from these areas was blood-stained. On other parts of the skin where the rash had been confluent the epidermis was stripping off leaving the surface raw. A putrid odour was observed. The trachea had small, ulcerated lesions extending down to the bronchi. The lungs were apparently normal except for a small portion of the upper lobe of the right lung. This appeared to be consolidated. Heart firmly contracted with somewhat excessive fat under the epicardium, otherwise normal. Aorta: Some athero-sclerosis. Liver large, somewhat congested, and on section looked like a nutmeg liver. Spleen smaller than normal, firm and fibrous. Kidney normal in size. Capsule stripped easily, substance rather pale, vessels more prominent than normal. Suprarenals normal. Bladder normal. Uterus: 2 small subserous fibroids. Intestines normal, but filled with a very large amount of faeces, especially the sigmoid and rectum.

CASE 4. Infant born with scabbing eruption; died 4 days after birth. General appearance normal save for eruption. Mouth, larynx, trachea normal. Lungs presented an unusual appearance; they were reddish-brown in colour, and on the surface was scattered small, white areas about 2 mm. in diameter. These areas extended for a short distance into the surface of the lung. The lungs were partially solid. Liver was dark red in colour and presented an appearance similar to that of the lung. Spleen apparently normal. Kidneys: There were small haemorrhages under the capsules and extending into the cortex. Suprarenals normal. Heart and circulatory system normal.

CASE 5. Infant born with alastrim. Mouth showed rash on the inside of the cheek. Larynx and trachea normal. Lungs and liver: condition similar to those of Case 4. Spleen and other organs normal.

*Inoculations.* Fluid was collected from the skin lesions of patients in various stages of the disease, and twenty-six rabbits and

four calves were inoculated by Professor MacCallum and myself. The skin was shaved and scarified, in some cases drawing blood, and the material was well rubbed in. These all gave negative results.

Blood from early cases and from a few patients who after contact with cases had developed headache and fever, but none of whom subsequently had an alastrim rash, was also used, but with negative results.

#### REFERENCE

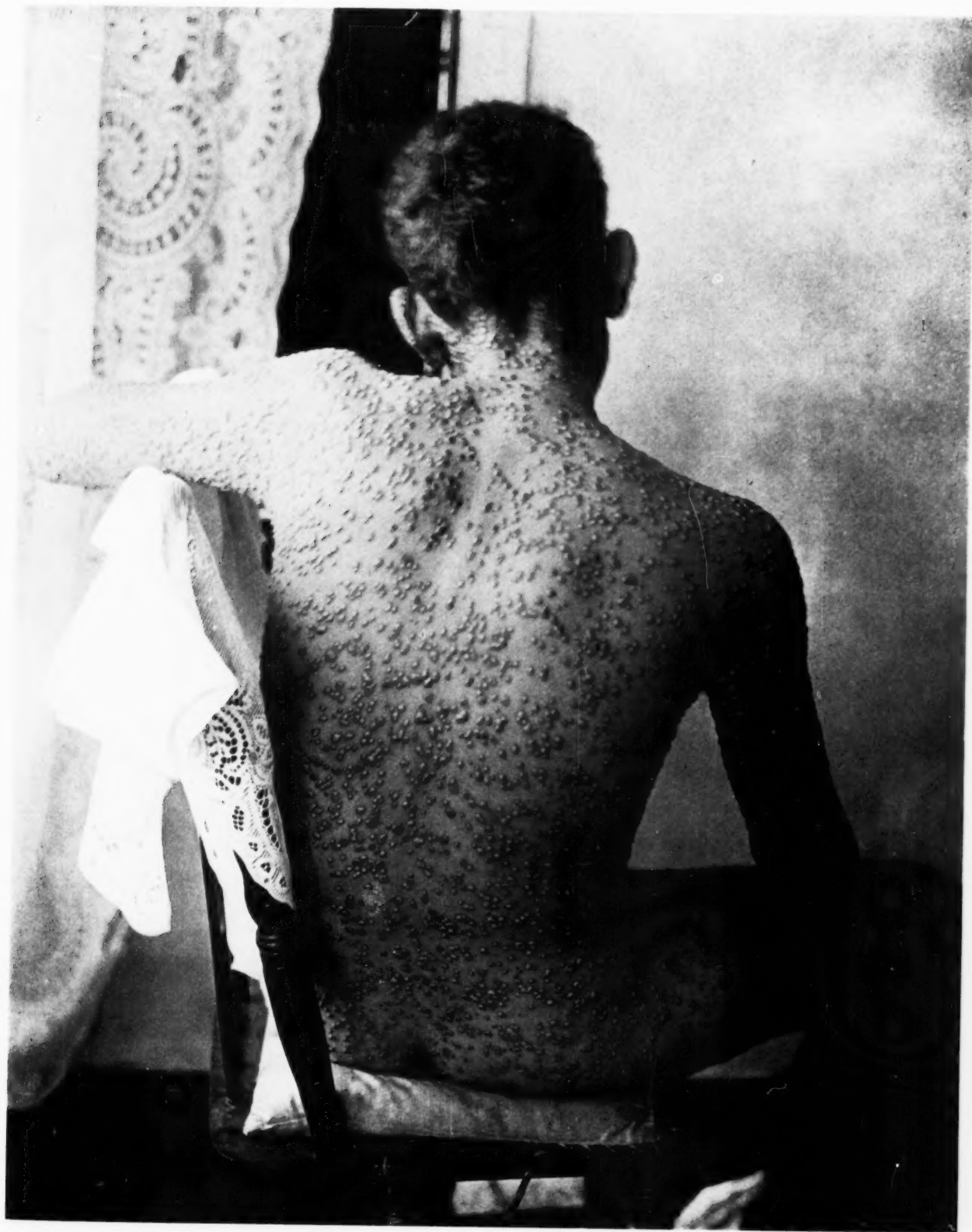
GOLDSMITH and LOUGHNAN (1921). *Journ. R.A.M.C.*, Vol. XXXVI, p. 66.



## EXPLANATION OF PLATE III

Severe case. Patient vaccinated twelfth day after exposure to infection. Rash developed on eighteenth day and ran concurrently with vaccination. Some of the lesions show secondary umbilication due to resorption of fluid.





#### EXPLANATION OF PLATE IV

Showing distribution of rash in a moderate case.



## EXPLANATION OF PLATE V

Showing effect of irritation. Note patches on inner side of left thigh above knee. This crop came out around a septic cut.



EXPLANATION OF PLATE VI

Eighth day of rash. Left eye almost closed.





## EXPLANATION OF PLATE VII

Child born at full term with well developed rash.  
Mother vaccinated one month previous to birth of child.  
No history of illness in mother. Wassermann reactions of  
mother and child negative.





## A NEW SPECIES OF *PHLEBOTOMUS* FROM TRINIDAD

BY

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*Phlebotomus trinidadensis*, sp. n.

*A relatively small species. ♂ genital armature with five large spines to the superior claspers: three terminal or distal, and two slightly beyond the middle distance, arranged with their bases on opposite sides of the segment; no tufts of non-deciduous hairs, proximally.*

*Colour* of both sexes similar. Pale ochraceous. Wings with the costa sometimes distinctly infuscated, and in certain lights with intense iridescent blue. Legs silvery grey.

*Male.* Abdominal hairs of the medio-dorsal line arranged in small, sparse groups on all of the segments; those of the venter dense, some of them semi-erect, others procumbent. Hairs of the proximal segments of the superior claspers very long and dense. *Palpi* of five segments: second, third, and fourth equal in length; the third and fourth broadened distally; fifth, two and a half times longer than the fourth. *Antennae* with the third segment projecting slightly beyond the tip of the proboscis; geniculated spines relatively very small, and apparently bilateral, those on the third about one-eighth of the entire length of the segment; those on the sixth and seventh a little less than one-fourth the entire length of the segments respectively. *Wings* (fig. 1 *a*) moderately narrow, the fork of the fourth vein generally in advance of the proximal fork of the second. *Genital armature* (fig. 1 *b*), relatively large; superior claspers each with five long, stout spines: three distal and two slightly beyond the middle distance, the latter arranged with their bases on opposite sides of the segment, the two outer, distal ones and the inner,



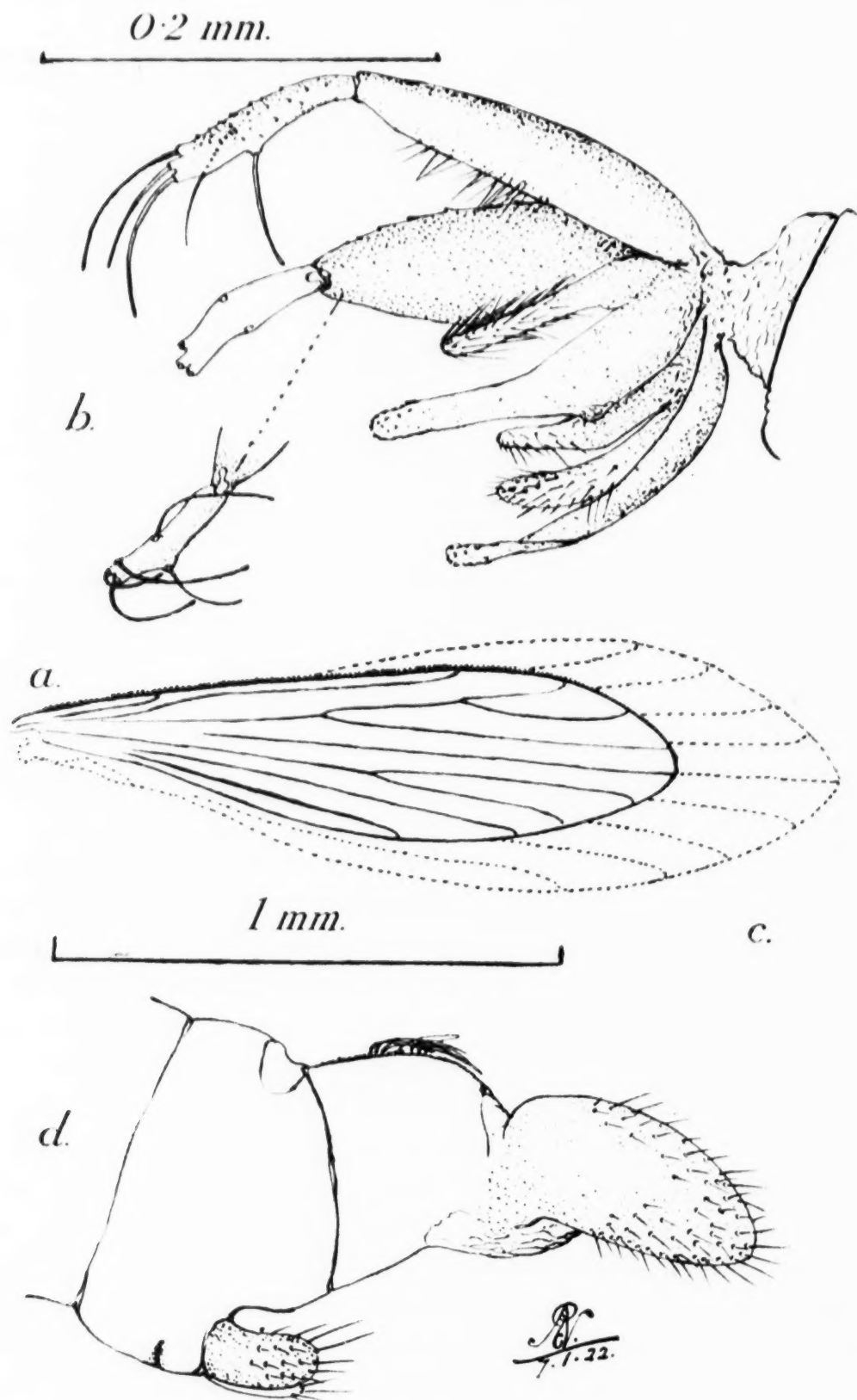


FIG. 1. *Phlebotomus trinidadensis*, sp. n.

♂ : *a*, wing; *b*, genital armature. ♀ : *c*, wing; *d*, terminal segments with the paired leaf-like appendages. *a*, *c* and *d* to same magnification.

lateral one longer than the others; the middle, distal one shortest; the segment about half the length of the proximal one. Inferior claspers slightly shorter than the proximal segment of the superior claspers.

*Length* 2.3 mm.; length from front of thorax to end of armature 1.8 mm.; wing, 1.3 mm.; leg III, 2.6 mm.; internal genital armature, 0.6 mm.

*Female*. More robust and generally larger than the ♂. *Abdominal hairs* more or less erect. *Palpi* similar in form to those of the ♂. Third segment of *antennae* shorter than the corresponding segment in the ♂, and not reaching the tip of the proboscis. *Wings* (fig. 1 c) much more broadly lanceolate than in the ♂; curvature of the borders similar; venation similar to that of the ♂. *External genitalia* (fig. 1 d): the superior leaf-like appendages relatively exceptionally large and, in macerated specimens, widely separated from the inferior pair; both appendages strongly hairy; the inferior pair with the finer hairs on the distal two thirds arranged in distinct, equidistant rows.

*Length*, 2.1 to 2.9 mm.; length to front of thorax, 1.7 mm.; wing, 1.6 mm.; leg III, 3.1 mm.

Two American species: *P. vexator*, Coquillett (1907) and *P. brumpti*, Larousse (1920), resemble this species in regard to the number of spines on the superior claspers. In *brumpti*, however, the armature generally resembles that of *P. papatasii*, and is, therefore, markedly distinct. *P. vexator* also differs in having the spines arranged as follows: two apical, *two sub-apical*, and *one in the middle* of the segment. In *P. trinidadensis*, sp. n., the formula is three apical and *two* near the middle of the segment.

TRINIDAD. Six ♂♂, seven ♀♀ (four of which contained blood), 1921. Major W. F. M. Loughnan, R.A.M.C., D.A., D.P., West Indian Command, with the assistance of Captain D. A. MacDougall, M.C., R.A.M.C.

In his letter from Kingston, Jamaica, dated 22nd August, 1921, Major Loughnan states that he, together with his fellow-officer, had a good deal of trouble in finding the specimens, and further that the species appeared to be very sparse in its distribution in the Island of Trinidad.

This is the first authentic record of the occurrence of a species

of *Phlebotomus* from the West Indies; and the captors are to be congratulated on their interesting discovery. Possibly other new and undescribed species await the hunters of these small midges in that region.

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## A NEW TSETSE-FLY FROM THE SOUTH CAMEROONS

BY

Professor R. NEWSTEAD, F.R.S.

AND

Miss ALWEN M. EVANS, M.Sc.

(Received for publication 10 February, 1922)

In the course of his investigations in the South Cameroons, during the past year, Dr. J. Hanington, a former member of the Staff of this School, made several small collections of tsetse-flies and other blood-sucking Arthropods. These he has generously presented to this Institution for the Museum collections. In the last consignment, which reached us towards the end of January of this year, were many examples of *Glossina palpalis*, R. D., *G. pallicera*, Bigot, and four specimens of a large species which, on microscopical examination of the morphological characters of the genital armatures, proved to be new and undescribed. In the letter accompanying the collection of flies, Dr. Hanington submitted a sketch-map of the districts through which he had passed, and gave the following brief account of the nature of the country in which the captures were made: 'The tsetse-flies were collected on my just completed tour of inspection N.W. over our border to Obudu. The country is hilly, forested, with many swift, shallow rivers, and full of tsetse. The large species is found only in the neighbourhood of Basho, where the ground begins to rise to the north into fly-free mountain-plateaux. The greatest number of tsetses were on the Mbilesi-Mateni Road, which runs along a wooded and rocky river valley.' The commonest species in this region would appear to be *Glossina pallicera*, of which twenty-six specimens were sent.

We append a description of the new species, and have ventured to dedicate it to Dr. Hanington, the discoverer, in recognition of his devotion to the science of tropical medicine.

*Glossina haningtoni*, sp. n.

*A large dark-coloured species, with infuscated wings, belonging to the 'Fusca Group.' Hairs of the third antennal segment relatively short. Proboscis (palpi) 0.7 to 0.9 mm. shorter than in G. FUSCA. Width of front in both sexes similar. Harpes of male each with three processes, the distal one angular and emarginate in front. Signum of female with height slightly exceeding width and paired crescentic folds almost continuous behind.*

*Male:* Length, 11 mm.; proboscis, 4 mm.; front of head, 0.75 mm.; wing, 11 mm. *Female:* Length, 11 to 12 mm.; proboscis, 4.2 mm.; front of head, 0.75 mm.; wing, 12 mm.

*Male:* Head with the posterior surface 'mouse-grey' (Austen), with a narrow black streak on the upper surface bordering the narrowly pale margin of the eyes. Vertex immediately behind the ocelli with a narrow black area. Front pale brown with a much paler area surrounding the ocelli. Antennal cavity greyish below, sides a little paler than the front. *Antennae* with the first two segments dark brown; the third pearly-grey, the tip of the segment moderately prominent, with the outstanding hairs forming the fringe in front from one-seventh to one-eighth the width of the segment. *Proboscis* relatively short, bulb uniformly pale buff-yellow. *Thoracic* markings very dark and pronounced, suture and ground colour forming the trident-like marking immediately in advance of the scutellum, pale ochraceous, the rest darker. *Abdomen:* Dorsum of first and second segment brown; the rest very dark, glossy sepia-brown, distal angles of last three segments ochraceous-grey; venter orange-ochraceous. Legs orange-ochraceous: leg I, with the femur infuscated along the dorsal half, tibia infuscated externally, tips of last two segments of tarsus dark brown or black; leg II, similar to the first but lighter in colour; leg III, with the third and fourth segments of the tarsi dusky, the last two all dark brown or blackish; all the ventral hairs dark golden. *Wings* rather strongly infuscated. *Genital armature* (fig. 1): Harpes (*h.*) with three bi-lateral processes; proximal pair long and spine-like, the first slightly shorter than the second; distal process angular, and when flattened by pressure shows a deep emargination on the distal margin (*h. 1*), but with the lower,



angular projection folded inwards the emargination almost entirely disappears (*h. 2*). Ventral chitinous sclerites long and projecting almost as far as the distal processes of the harpes. Inferior claspers (*i. c.*) normal, a few of the marginal hairs of great length. Median process with its distal edge rounded, and projecting slightly beyond the inferior claspers. Superior claspers (*s. c.*) relatively rather long, and as usual, bluntly bifid.

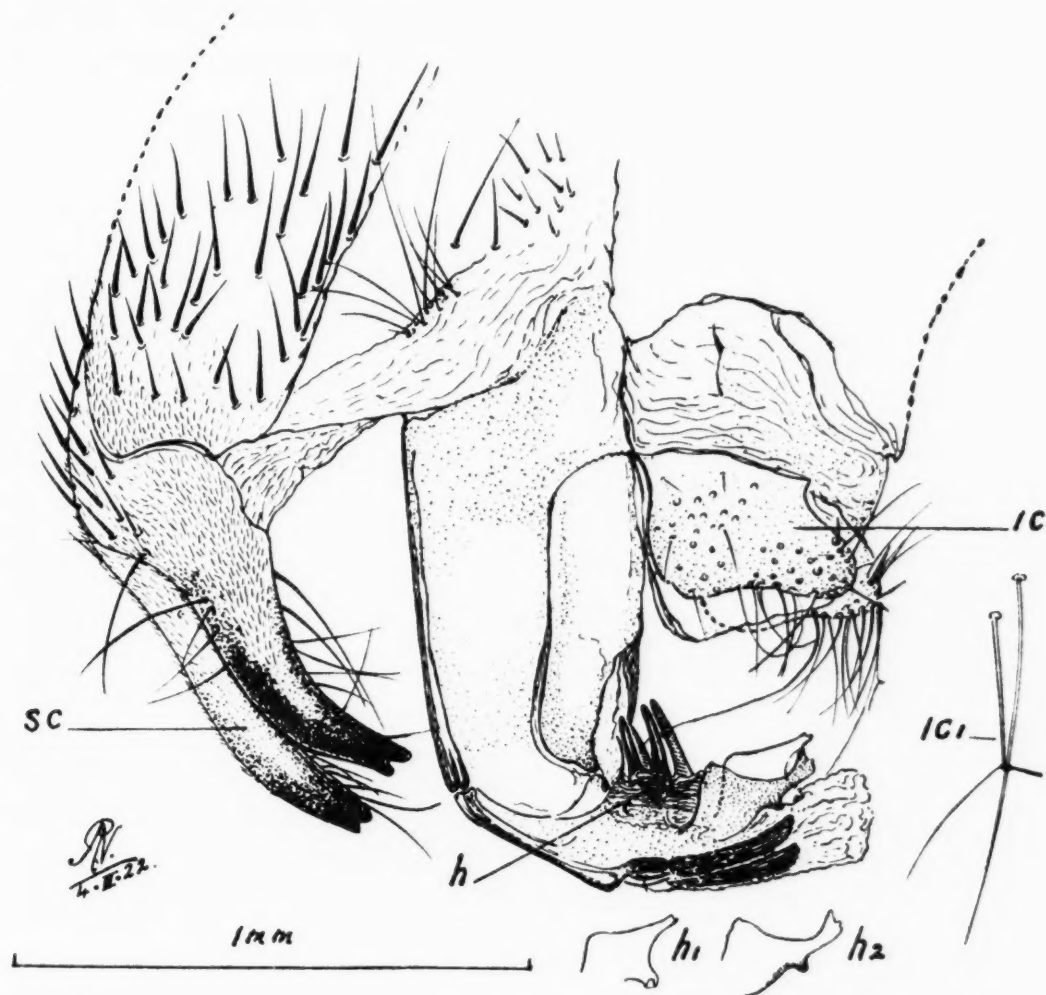


FIG. 1. *Glossina baningtoni*, Newstead and Evans. ♂ genital armature: *s.c.*, superior claspers; *i.c.*, inferior claspers; *i.c.l.*, two hairs from the inferior clasper, one of them malformed; *h*, harpes; *h.1*, distal process of harpe, with lower arm extended, internal aspect; *h.2*, the same with the lower arm curved inwards, external aspect.

*Female*: Third antennal segment pale ochraceous proximally, the distal three-fourths infuscated. Colour of legs, abdomen and plurae slightly darker than in the male. The 'black streak' on the posterior surface of the head absent. The space between the eyes (front) as in the male. *Genital armature*: External armature of the type found in *Glossina fusca* but the dorsal plates rather broad, the

width exceeding one-third of the length. Internal armature, signum of uterus (fig. 2) measuring 0.41 mm. in height and 0.38 mm. in greatest width. Median portion of signum (*m. p.*) a thin plate of the form shown in the figure, ochraceous brown behind, becoming straw-coloured towards the anterior margin; postero-lateral portions (*p. l. p.*) laminar, pale ochraceous, connected with the median plate by deep crescentic folds of black chitin (*c. c.*) These folds almost continuous posteriorly, and forming a striking feature of the signum.

SOUTH CAMEROONS: Basho, Mamfe (Ossidinge) Division, 14th December, 1921, 2 ♀♀; 2 ♂♂. Dr. J. Hanington.

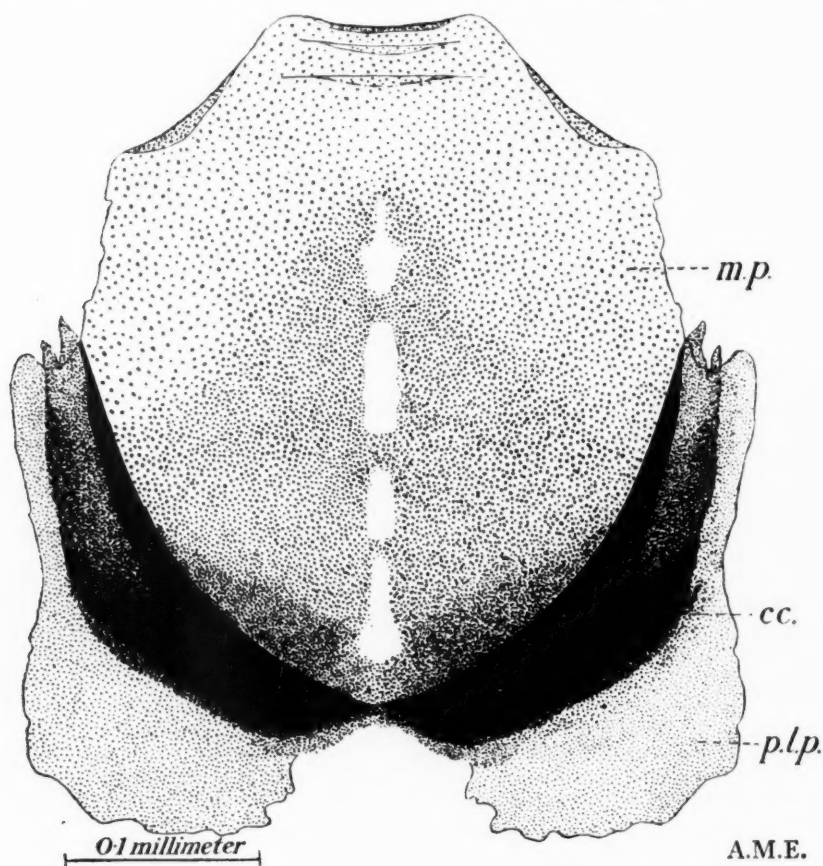


FIG. 2. *Glossina baningtoni*, Newstead and Evans. ♀ Signum: *c.c.*, crescentic fold; *m.p.*, median plate; *p.l.p.*, postero-lateral plate.

Closely related to *Glossina fusca*, but differing *externally* by the relatively much shorter palpi (proboscis), and the slightly more robust appearance. But the most marked morphological differences can be seen only in the genital armature of both sexes. A careful study of these organs at once reveals the strikingly distinctive features of this species, and its affinities with other members of the 'Fusca Group' of tsetse-flies.

## NOTES ON AUSTRALIAN CESTODES

BY

P. A. MAPLESTONE

*(Received for publication 10 February, 1922)*III. *COTUGNIA OLIGORCHIS*, n. sp.

On four occasions specimens of the cestode about to be described were found in the intestine of the Whistling Duck (*Dendrocygna arcuata*, Cuvier), shot a few miles from Townsville, North Queensland.

## EXTERNAL ANATOMY.

The largest specimen measured 80 mm. long and 8 mm. broad at its widest part; these dimensions were taken from fixed material.

The scolex is relatively small and there is no neck. In well fixed specimens the worm is of almost uniform breadth for the greater part of its length, but tapers fairly rapidly and evenly both anteriorly and posteriorly. The posterior end is not unlike the anterior, except that it is not so finely pointed, owing to the absence of a scolex.

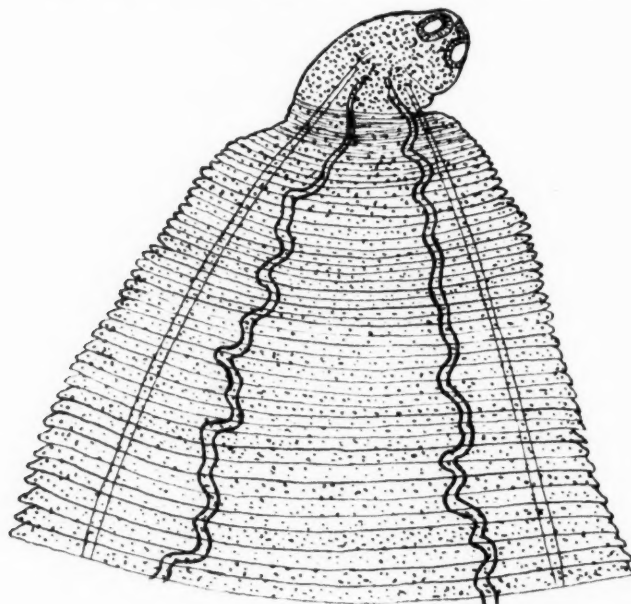
*Head.* The scolices were not well fixed, being in all cases more or less shrunken, so the detailed characters of this structure cannot be accurately given. However, it is seen to bear a very small retractile rostellum armed with a single row of minute hooks measuring about  $10\mu$  long, but unfortunately their exact number could not be determined, because all available specimens were imperfect. The four small suckers are situated quite near the anterior extremity, and measure about  $0.65\mu$  in diameter (fig. 1).

*Segments.* The proglottides are from first to last much broader than long.

## INTERNAL ANATOMY.

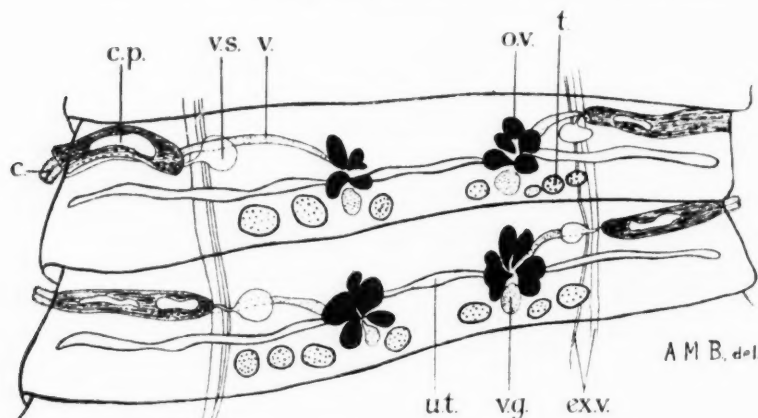
*Muscular system.* On examining transverse sections it is seen that the muscle layers are disposed in the same way as in *Diploposthe laevis* (Bloch, 1872); Jacobi, 1896. That is, there are a few diagonal fibres externally, with a layer of transverse fibres internal to them. Next in order from without inwards is the main longitudinal layer, which consists of a large number of closely set

bundles oval in cross section, with their long diameters running dorso-ventrally, and about  $0.50\mu$  in thickness. This layer is evenly developed and encircles the segment, except where it is pierced by



A.M.B. del.

FIG. 1. *C. oligorchis*, n. sp. Scolex and anterior portion of strobila.  $\times 35$ .



A.M.B. del.

FIG. 2. *C. oligorchis*, n. sp. Mature segments. *c.*, cirrus; *c.p.*, cirrus pouch; *ex.v.*, excretory vessels; *ov.*, ovary; *t.*, testes; *ut.*, uterus; *v.*, vagina; *v.g.*, vitelline gland; *v.s.*, vesicula seminalis.  $\times 20$ .

a cirrus pouch. Internal to this is another thinner layer of transverse muscle with a few scattered bundles of longitudinal muscle irregularly placed on the dorsal and ventral surfaces respectively, and about fifteen to twenty in number on each surface. Internal to these bundles are a few fibres of transverse muscle. It should be noted that the transverse muscle layers consist of hoop-like strands of fibres discontinuous with each other antero-posteriorly, so that in transverse sections they are only seen here and there. (Figs. 3



and 4 do not show the transverse fibres for this reason.) The dorso-ventral fibres are most marked in sections through the anterior and posterior of a segment.

*Nervous system.* The nervous system is poorly developed and consists of a small main nerve lying well to the outer side of the excretory canals, and ventral to the cirrus pouch and vagina.

*Excretory system.* The two lateral excretory canals on each side lie in the anterior portion at some distance from each other; the smaller dorsal vessel lies to the inner side of and dorsal to the ventral vessel. In this part of the worm the dorsal canal pursues a

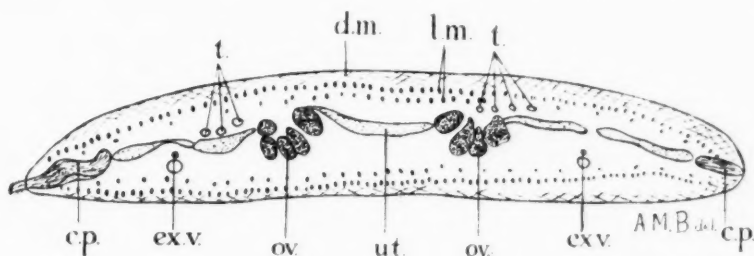


FIG. 3. *C. oligorchis*, n. sp. Transverse section through a mature segment. *c.p.*, cirrus pouch; *d.m.*, diagonal muscle; *ex.v.*, excretory vessels; *l.m.*, longitudinal muscle; *ov.*, ovary; *t.*, testes; *ut.*, uterus.  $\times 20$ .

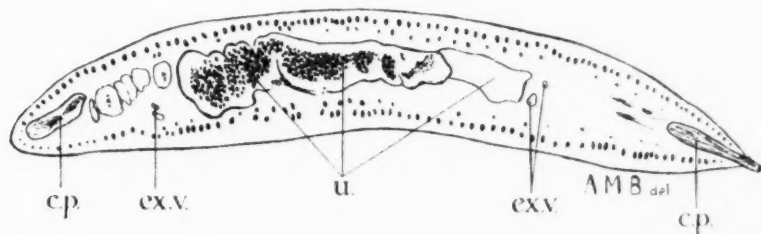


FIG. 4. *C. oligorchis*, n. sp. Transverse section through a gravid segment. *c.p.*, cirrus pouch; *ex.v.*, excretory vessels; *u.*, uterus.  $\times 20$ .

wavy course, each wave extending over two or three segments, so that the width of the medulla varies slightly in different segments (fig. 1). More posteriorly it straightens out like the ventral vessel and pursues a direct antero-posterior course, and at the same time comes to lie close to the inner side of the latter. Throughout their whole length the canals are a considerable distance from the lateral borders of the worm.

*Genitalia.* The male and female organs lie in the medulla in two separate groups, one on each side of the mid-line.

*Testes.* The testes vary from three to five in number on each side and are situated posterior to the ovary, close to the posterior border of the segment in a transverse line. They measure about

80 $\mu$  in diameter, but this dimension is only approximate, for when five are present they are smaller than when only three are present on each side. Their number and position in relation to the ovary (whether lateral or mesial to it) vary in different proglottides of the same chain, or even on the two sides of the same segment. Thus when there are three testes they may all lie external to the ovary, or there may be one internal and two external; if four in number they may lie, two internal and two external, or three external and one internal; and lastly, if five in number they may lie three external and two internal, or four external and one internal (fig. 2). They come to full development far in advance of the ovary, and are beginning to atrophy before this organ is fully developed.

*Vas deferens.* There is a small but distinct vesicula seminalis, which lies just internal to or overlapping the excretory canals; from its outer side a narrow, lightly coiled tube leads to the base of the cirrus pouch which it enters. It runs on the dorsal side of the excretory canals. The cirrus pouches are long and relatively thick saccular organs lying transversely near the anterior margins of the segments. In the early stages of development, the cirrus pouches on each side lie to the inner side of the excretory canals, but they soon pass to their outer sides, which relation they then maintain to the end of the chain. They are about 630 $\mu$  long and 110 $\mu$  broad, and open into small chambers which in turn open in distinct pores situated on the lateral borders, not far from the anterior lateral angles. The cirri are often seen partly extruded through these pores, and they are relatively thick and straight, being of the same diameter for their whole length, with slightly rounded tips. They are about 460 $\mu$  long and 45 $\mu$  in cross section, and their outer surfaces are thickly covered from base to tip with small straight spines set perpendicular to the surface and about 7 $\mu$  long. These organs are the same in appearance and have the same relations with the other organs on both sides of each segment (fig. 2).

*Ovary.* The paired ovaries are large and are situated one on each side of the mid-line, about mid-way between the anterior and posterior borders of the segments. Each consists of four or five lobes, which radiate forwards and laterally from a central point; the small compact vitellarium lies close behind them. The distances of the ovaries from one another, and consequently from the lateral



borders on the corresponding sides, vary slightly in different segments. The shell gland, as a rule, is not clear, but in some segments it can be seen lying between the ovary and vitelline glands (fig. 2).

*Receptaculum and vagina.* The vagina is a relatively wide tube; running from the ovary it first curves forwards and outwards, then turns and runs directly outwards, and crossing ventral to the seminal vesicle, but dorsal to the nerve and excretory canals, it finally runs ventral to the cirrus pouch to open at the genital pore on the ventral side of this organ. The final part of its course can only be determined in sections.

*Uterus.* The uterus is visible at an early stage as a thin transverse tube crossing the proglottis almost from one side to the other, about mid-way between the anterior and posterior borders of the segment (fig. 2). As it develops, it throws out numerous branches in every direction, which gradually increase in size, so that eventually the uterus appears as a broad saccular organ occupying nearly the whole of the segment, with a few trabeculae representing

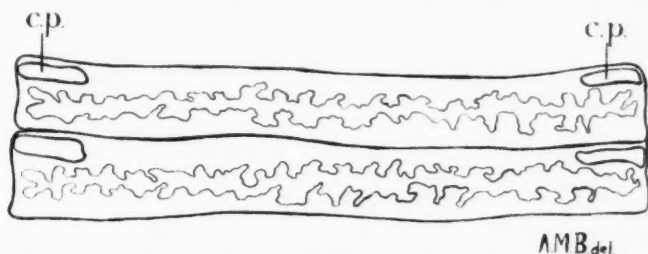


FIG. 5. *C. oligorchis*, n. sp. Uterus in intermediate stage of development. c.p., cirrus pouch.  $\times 20$ .

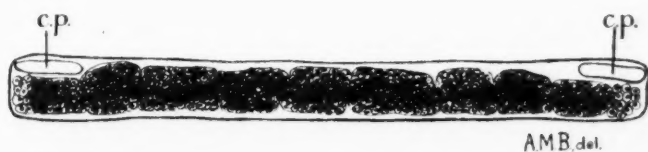


FIG. 6. *C. oligorchis*, n. sp. Fully developed uterus. c.p., cirrus pouch.  $\times 17.5$ .

the remains of the original branches (figs. 5 and 6). When the uterus is fully developed, the eggs lie singly in capsules.

In sections showing the early stages of the uterus, it is seen to pass between the testes (dorsal) and the ovaries (ventral), and at the sides it crosses the excretory canals dorsally and runs almost to the edge of the segments (fig. 3). Later, it pushes the canals ventrally, and tends somewhat to disturb the contour of the muscle layers

(fig. 4). No fully developed eggs were seen, the most mature ones measured about  $43\mu$  and the oncosphere  $26\mu$  in diameter.

#### DIAGNOSIS.

Up to the present, ten species of the genus *Cotugnia* have been recorded (Meggitt, 1920). All except *C. browni*, Smith, possess numerous testes. *C. browni* has six to seven testes, but these lie anterior to the female glands. The present species possesses only from three to five testes on each side, and these lie posterior to the female glands; it is thus obviously new, and is accordingly named *Cotugnia oligorchis* on account of the few testes.

The type specimens are in the museum of the Liverpool School of Tropical Medicine.

NOTE.—*Diploposthe laevis*, Bloch, was first recorded in Australia by Krefft under the name *Taenia tuberculata*; this material was re-examined by Johnston (1912), who assigned it to the above species. The host, in this case, was *Aythya australis*, Gould, the White-eyed Duck or Widgeon. Later on, Johnston (1913) recorded the same cestode in Queensland; this time the host was *Dendrocygna arcuata*, Cuvier, and his specimens came from the Australian Institute of Tropical Medicine. The writer, on examining the slide of this cestode, placed in the Institute museum by Johnston, found that beyond doubt it is a worm of the above described species with two ovaries, and is not *D. laevis*. Therefore the record by Johnston of *D. laevis* in the host *D. arcuata* is not correct. However, *D. laevis* does exist in Queensland, for the writer has recently examined some material at the Australian Institute which proved to be *D. laevis*; these worms were taken from *A. australis*, the original host in which Krefft found it in New South Wales.

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## NOTES ON AUSTRALIAN CESTODES

BY

P. A. MAPLESTONE

AND

T. SOUTHWELL

*(Received for publication 10 February, 1922)*IV. *GYROCOELIA AUSTRALIENSIS*, Johnston

This cestode is evidently fairly common, as it was found in the intestines of several members of the species Spur-winged Plover (*Lobivanellus lobatus*, Lath.) shot in the neighbourhood of Townsville, North Queensland.

## EXTERNAL ANATOMY.

Fixed worms measured 167 mm. in length, with a maximum breadth of 4 mm.

The worm is very narrow anteriorly, and widens fairly rapidly and evenly posteriorly. The most striking character is its dorso-ventral diameter, which is very great, especially towards the posterior end. The segments are thick in the centre and thin at the edges, so that in cross section they are bi-convex. The large regularly alternating cirrus, extruded in most mature segments, can be easily made out with the naked eye.

*Head.* The scolex is flat anteriorly, and measures 0.315 mm. in breadth and 0.22 mm. in length. From the centre of the anterior surface arises a thin rostellum about 120 $\mu$  long and 40 $\mu$  broad, tapering anteriorly and ending in a bluntly rounded tip; there is very little muscle in this organ. Unfortunately, in all our specimens the hooks had been lost. The four suckers are placed, two on the dorsal and two on the ventral surface of the scolex, and look directly dorsally and ventrally respectively. They are circular in outline, and measure about 130 $\mu$  in diameter. Behind the scolex there is no true neck, but a short unsegmented portion of about the same width (fig. 1).

*Segments.* Segmentation begins at a distance of 4.8 mm. from the anterior end, and about the first ten segments become successively narrower, thus giving rise to the appearance of a neck. The minimum breadth is about  $170\mu$ ; from this point the segments progressively increase in width to the posterior extremity.

The dimensions of mature segments are 1.25 mm. across the anterior, and 1.5 mm. across the posterior borders, with a length of 0.8 mm. It is thus apparent that the posterior angles are slightly projecting (figs. 2 and 3).

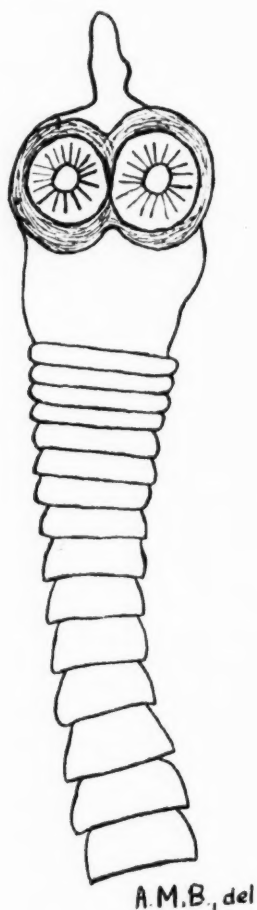
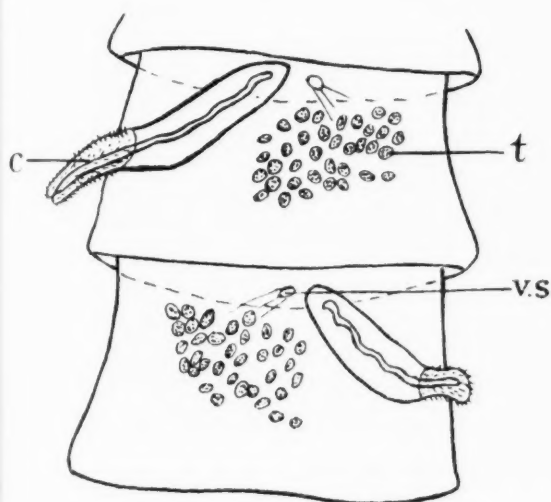


FIG. 1. *G. australiensis*. Scolex and anterior portion of strobila.  $\times 56$ .

#### INTERNAL ANATOMY.

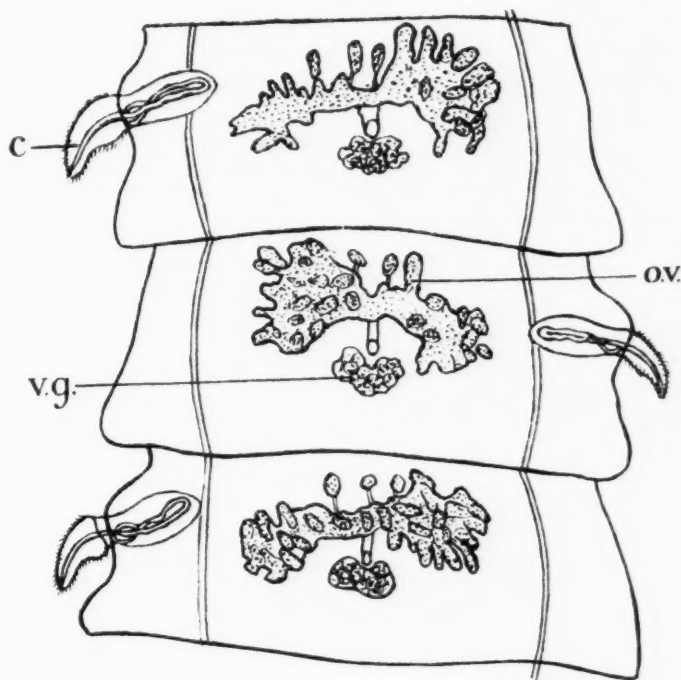
*Muscular system.* In transverse sections the relatively great thickness of the cestode is seen to be due chiefly to the longitudinal muscle fibres, which are arranged in two distinct layers (fig. 4). From without inwards the structures are arranged as follows. First there is the cuticle, which is about  $90\mu$  thick, then a layer of transverse muscle, and next to it the outer layer of longitudinal

muscle. This measures about  $60\mu$  in thickness, and is composed of oval, discrete bundles of muscle fibre lying with the long axis of the bundles dorso ventral. On the inner surface of this layer is another thin band of transverse muscle, which has on its inner surface the



A.M.B., del.

FIG. 2. *G. australiensis*. Young segments showing male genitalia. c., cirrus; t., testes; v.s., vesicula seminalis.  $\times 35$ .



A.M.B., del.

FIG. 3. *G. australiensis*. Older segments showing female genitalia. c., cirrus; ov., ovary; v.g., vitelline glands.  $\times 35$ .

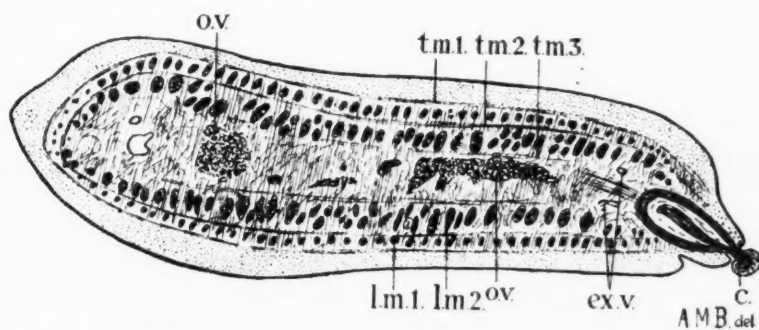


FIG. 4. *G. australiensis*. Transverse section of mature segment. c., cirrus; ex.v., excretory vessels; lm.1, outer layer of longitudinal muscle; lm.2, inner layer of longitudinal muscle; ov., ovary; tm.1, outer layer of transverse muscle; tm.2, middle layer of transverse muscle; tm.3, inner layer of transverse muscle.  $\times 20$ .

second and thicker layer of longitudinal muscle. The dorso-ventral diameter is about  $140\mu$  in the mid-line, and gradually decreases towards the sides; like the outer layer it is composed of oval bundles of fibres, but in many cases these are broken up into smaller



subsidiary bundles. Dorso-ventral fibres can be made out running between the bundles of longitudinal muscle. On the inner side of the second layer of longitudinal muscle are a few scattered transverse fibres, and these with the two outer layers of the same fibres end in the outer coat of the cirrus pouch where this organ is present.

*Nervous system.* The main longitudinal nerve is situated well to the outer side of the ventral vessel, and ventral to the cirrus pouch. Further details of this system were not investigated.

*Excretory system.* The dorsal-lateral excretory vessel is smaller in diameter than the ventral, and lies directly dorsal and close to it, except when the cirrus pouch passes between them, where they become more widely separated.

*Testes.* The testes are found only in young worms in which there is no trace of the female genitalia, even in the terminal segments. They first appear about the thirtieth segment, and from this point posteriorly they gradually become more developed, and again dwindle, until at about the hundredth segment they have quite disappeared. The testes number about fifty in full development, and measure  $45\mu$  on an average. They occupy the central portion of the proglottides (fig. 2).

*Vas deferens.* The vasa efferentia appear to unite in a small globular structure, evidently a vesicula seminalis, which lies in the anterior of each proglottis, near the middle of the segments and just at the mesial end of the cirrus pouch. They are in no instance conspicuous, and do not appear to function for storing the spermatozoa, except in the earliest stages; as the cirrus develops, the vesicula atrophies and finally disappears. That portion of the vas deferens within the cirrus pouch becomes increasingly coiled as the seminal vesicle atrophies, and it appears to take on the functions of the latter.

*Cirrus pouch.* The cirrus pouch is a relatively large, thick-walled sac, lying diagonally across the antero-lateral angle of the segments on the pore side, and it opens with absolutely regular alternation on the lateral borders of the segments, slightly in front of the middle. It measures about  $450\mu$  long and  $150\mu$  broad, and as the worm is at this time only about 1 mm. broad, it is a conspicuous organ. It persists in older worms after the testes are gone and the female glands are well developed; but at this stage



the worms are about 2 mm. broad, and as the cirrus pouch does not increase above the dimensions given previously, its size in relation to that of the proglottides is not so great.

The cirrus is nearly always extruded; it measures about  $400\mu$  long and  $90\mu$  thick at the base. In the early stages it is a long, fairly thick tubular structure, slowly tapering from base to bluntly rounded tip. Its external surface is thickly covered with backward-curving spines about  $4\mu$  long (fig. 2). It runs posteriorly as a rule, and in many cases is recurved, so that the tip points towards the lateral border of the same or the succeeding segment. In worms in which the female genitalia are developed, the cirrus is generally stouter and more conical in shape, and it tapers much more rapidly (fig. 3).

*Ovary.* The ovaries are only found in older worms. They first appear about the seventy-fifth segment, and have reached full development by about the ninety-fifth segment. In these worms the seventy odd small segments in front of the one in which the ovary is first seen are devoid of all traces of genitalia, either male or female. Apparently therefore, in attaining a certain age, the worms lose their power of developing reproductive organs, any further segments being sterile.

Fully developed ovaries are about  $700\mu$  broad and consist of two lobes each composed of lobules running for the most part laterally. The two lobes are united across the mid-line by an isthmus, they are unequal in size, the one on the cirrus side being only about half the size of the one on the opposite side. The result is that they present a regularly alternating asymmetry (fig. 3). In addition, there are three or four small masses of ovarian tissue lying anterior to the isthmus of the gland, and more or less directly connected with it. The ovaries lie across the centre of the proglottides about mid-way between the anterior and posterior borders.

*Receptaculum and vagina.* Both these structures are absent.

*Vitelline glands.* The vitelline glands consist of a small horseshoe-shaped mass of tissue, with the concavity facing forwards. They lie in the mid-line behind the ovaries and towards the posterior margin of the segments; ducts can be seen running forwards from their concavities, and these have the follicles of small shell glands grouped around them.

*Uterus.* The uterus first appears as an oval tubular ring nearly completely surrounding the ovary and vitellarium, which rapidly atrophy (fig. 5). Outpocketings soon appear on the tubular uterus (fig. 6). They become progressively larger and more complicated as development proceeds, until the ring-like structure is nearly lost, and in full development the uterus is represented by a large lobulated sac occupying almost the whole of the proglottides, both laterally and antero-posteriorly (fig. 7).

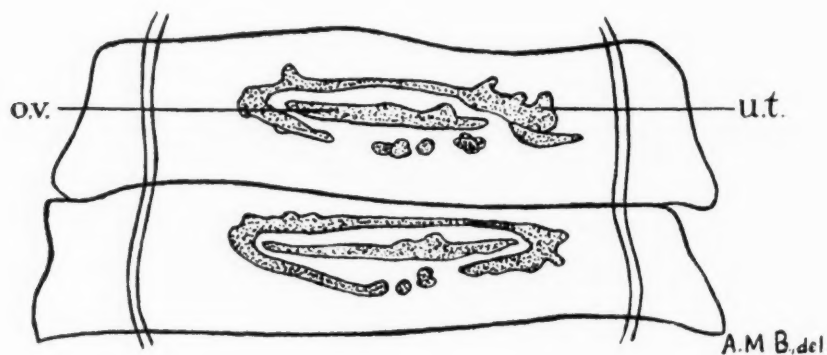


FIG. 5. *G. australiensis*. Segments showing first stage of uterus. *ov.*, ovary undergoing atrophy; *ut.*, uterus.  $\times 35$ .

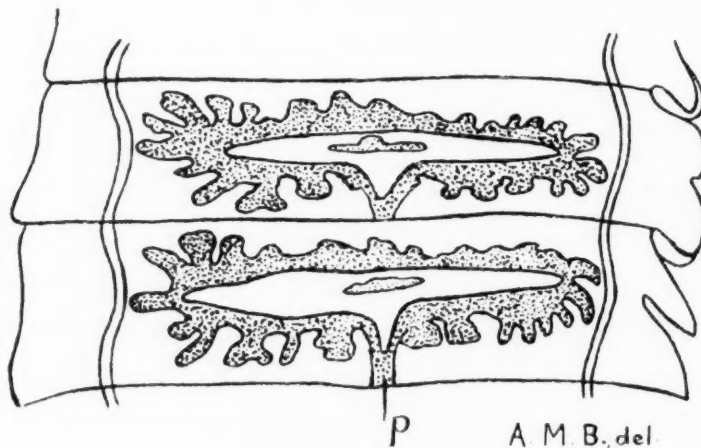


FIG. 6. *G. australiensis*. Segment showing uterus more fully developed. *p.*, uterine pore.  $\times 35$ .

Another remarkable development is that of uterine pores; these open externally on the centres of the posterior borders of the segments, one on the dorsal and the other on the ventral surface. From examination of horizontal sections, we are of the opinion that these pores arise from a single central opening on the posterior of the uterus, from which two canals run, one to each pore (figs. 6 and 7).

*Eggs.* The eggs are slightly oval with blunt extremities. They measure about  $65\mu$  long and  $52\mu$  broad, and the contained embryo, which is enveloped in an albuminous covering, is also oval, and measures about  $36\mu$  by  $26\mu$ . The hooks on the embryo are about  $16\mu$  long.

Fig. 7

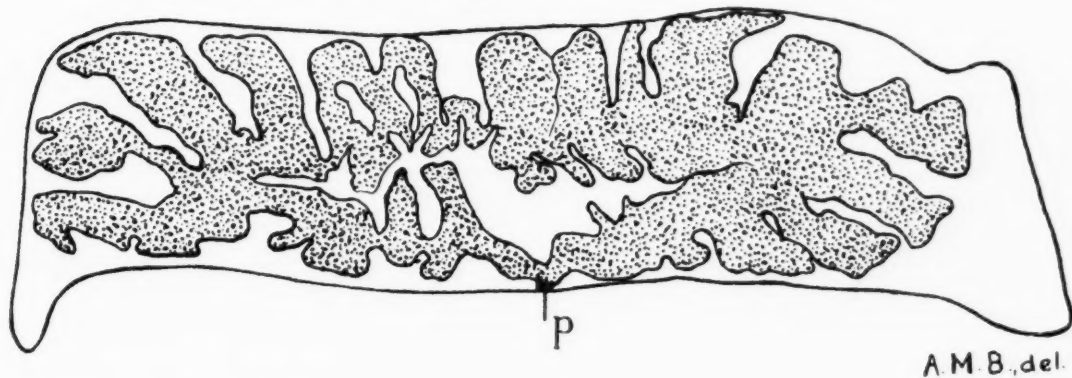


FIG. 7. *G. australiensis*. Fully developed uterus. *p.*, uterine pore.  $\times 35$ .

#### DIAGNOSIS.

Although the hooks had been lost from our specimens there seems no reason to doubt that the worm is *Gyrocoelia australiensis*, Johnston. It is, however, necessary to point out that Johnston (1912) figures five minute testes lying immediately anterior to the ovary. Our specimens present a similar appearance, but these structures seem to us to be detached ovarian acini. Further, Johnston (1914) recorded a *Gyrocoelia* sp. from *L. lobatus*, which worm he obtained from the Australian Tropical Institute, the same source as our material, so it is practically certain his unnamed species is also *G. australiensis*.

Clausen (1915), in his description of *G. paradoxa* (von Linstow) (= *Brochocephalus paradoxus*), figures a bi-lobed or double receptaculum seminis enclosed in the uterine ring. Our specimens present a somewhat similar appearance, but the structure is obviously the degenerate ovary and vitellarium.

The occurrence, in our worm, of the male and female sexual organs at different times, which results in a strobila being male when young and female when middle aged, raises the point as to whether

this condition does not likewise exist in the genus *Dioicocestus*, Führm., 1900, in which case its characters would be limited to the possession of double male genitalia, and an irregularly alternating vagina.

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# A CONTRIBUTION TO THE KNOWLEDGE OF THE BIONOMICS OF SAND-FLIES

BY

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## PLATE VIII

During the late campaign in Macedonia, members of the B.E.F., Salonika, suffered more or less continuously during the hot season from sand-fly bites and sand-fly fever. As seems to be always the case, the trouble was very definitely localised, the usual foci being either dilapidated villages in or near which units were stationed, or entrenched positions which had been long occupied. In the summer of 1918 there was a severe outbreak of the fever in and round Janes (where in one unit from mid-June to mid-August there was an incidence of 150 per cent. of the total strength), and I was instructed in the third week of June to investigate the conditions as regards sand-fly in the district. Owing to an attack of dysentery I was unable to proceed to Janes until the second week in August, when much valuable time had unavoidably been lost. The following pages reproduce almost verbatim a report on my investigations between August 12th and September 26th based on copious notes made on the spot. There have been incorporated also various observations made during 1917-1919 on *Phlebotomus* in Macedonia.

It may not be irrelevant to mention in passing that on my way to Salonika in July, 1917, I kept a constant lookout for *Phlebotomus* wherever the frequent stoppages of a troop train offered an opportunity of searching any likely spot. A species of the genus (probably *papatasi*) was first encountered at a small station near Beaune (Côte d'Or), and other examples were noted later at Orange (Vaucluse). At the time I was not prepared to find the genus so far north, but Major Joyeux has subsequently told me that during the war special search was made for *Phlebotomus* in France, with the result that *P. papatasi* was met with amongst other places near Beaune and as far north as Paris itself.

In Macedonia the species of sand-fly (*Phlebotomus*) investigated



were three in number, viz., *P. papatasi*, Scop.; *P. minutus*, Rnd., and *P. perniciosus*, Newst., the first named occurring, at Janes, in by far the greatest numbers. Owing to the difficulty of discriminating between the females of these insects no attempt was made to determine specifically all the material collected, but in some hundreds of cases in which identification was effected *P. minutus* formed about 1 per cent. and *P. perniciosus* 2 to 3 per cent. of the total. Probably one is safe in saying that 95 per cent. of the sand-flies observed belonged to *P. papatasi*. Melanic forms of all three species were observed and in long series of *papatasi* many colour variations were noted, but the proportion of these to the total was not worked out. Really dark examples were, however, uncommon. From various sources it appears that by the date on which observations were commenced sand-flies were much less common than earlier in the season (June). Nevertheless, up to September 10th they were numerous wherever sought for, and in certain localities very abundant. Between the 10th and the 14th of the month there was a great falling off in the numbers found daily, but even so a good many could be taken up to the end of the month, and these later captures were still persistent in their attacks.

In connection with the association together and seasonal prevalence of these sand-flies, it is interesting to note that in 1917 (August to September) I did not certainly meet with *P. minutus*, while *P. papatasi* and *P. perniciosus* (though one or other occurred in numbers at Kalamaria, Salonika, Karasouli, Lahana, Nigoslav) were never taken together. In the present season, 1918 up to June 24th, only *perniciosus* occurred sparingly at Kalamaria. Again between July 24th and August 10th *perniciosus* (with one or two *minutus* latterly) was so abundant in the same place that in a single evening (9 to 11 p.m.) two hundred could be taken at light in one latrine. *P. papatasi*, though carefully sought for, was not detected. Newstead, in describing *perniciosus*, records a similar experience in Malta. 'Two examples of *P. minutus* were found in association with this species, but strange as it may seem, not a single example of *P. papatasi* was either captured or seen on these occasions.'

In 1917 sand-flies (*P. perniciosus*) were still common at Karasouli in the third week of October.



As to the part played by these three species respectively in carrying sand-fly fever in Macedonia, little evidence is to hand. Assuming that all are potential vectors, it is by no means certain that each carries the fever in proportion to its numbers. In any case *P. minutus* seems hardly likely to be of much importance in this rôle. As regards *papatasi* and *perniciosus*, the former is in my experience not only more active but more voracious and incessant in its attacks. In three localities where sand-fly fever was reported (Karasouli, 1917; Salonika, Janes, 1918) it was present in numbers, and in two instances was the only sand-fly taken. My present impression is that *perniciosus* is if anything a less efficient vector than *papatasi*, but the point is one which can be settled only by direct experiment.

The work done at Janes has included the following:—(a) A search for the early stages of sand-flies *in situ*. (b) A study of the habitats and habits of the imago. (c) Breeding and rearing of the flies. (d) Preventive measures.

#### A. SEARCH FOR THE EARLY STAGES

During the whole of my stay at Janes search has been made more or less continuously for ova, larvae, or pupae of sand-flies. In the first three weeks practically nothing else was attempted, but the result of protracted examination has been negative. Search has been made (1) in the soil itself to a depth of 6 inches, particularly near any indication of moisture; (2) along the sides of earth cracks and fissures so far as they could be followed; (3) beneath loose stones and in the superficial layer of cracked rock at or near outcrops; (4) between sandbags and loose earthy rubbish thrown up in erecting tents, marquees, etc.; (5) between mud bricks or masonry in the lower tiers of buildings; (6) in the dampest looking recesses of dug-outs and among débris beneath hay stacks.

This failure to find any early stages is parallel to the experience of many other investigators, and does not necessarily imply that search was made in wrong directions. Subsequent experience of ova and larvae from captive females indicated that the earth, etc., previously examined was much too dry, and that larvae, if they were to be found, should have occurred at greater depths. It is

possible, however, that larvae are absent or much less plentiful while the adults are numerous. Newstead, who worked in Malta in July, August and the first week of September was under the impression that larvae would be more numerous in autumn about a week after the adult had disappeared. As has already been stated, sand-flies began to decline in numbers at Janes between the 10th and 14th of September, and it was on the 20th that I found newly-hatched larvae for the first time in my breeding-boxes (see below, App. III, No. 1).

## B. HABITATS AND HABITS OF FLIES

*Habitats.* (1) In mid-August, and for some time afterwards, sand-flies occurred practically everywhere in and near the C.C.S. They were more numerous, however, in tents and wards. In dug-outs they were comparatively scarce, which was at first rather surprising since at the same time one had a report that up the line the flies swarmed in dug-outs. The explanation appeared to be simply that the flies gathered as near as possible to their hosts. The hospital dug-outs were unoccupied, while the others were regularly used. In the same way where two wards, one occupied the other empty, adjoined, the former yielded many more flies than the latter. Again in a ward which, as sometimes happened, had only a corner bed occupied, the flies were most numerous in that corner. In large marquees or wards the distribution of the flies varied from day to day and with the hour of the day. After a windy night few could be found, and those that occurred were in sheltered corners. They were most abundant after still, damp nights. In the morning one found them mainly beneath the flaps running round the top of the sides of the tent, and the flies so taken showed a large percentage of newly-fed, gorged females. Later in the day, from roughly 11 or 12 a.m. to about 2 p.m., the flies became temporarily scarcer, while during the afternoon they were more frequently seen in numbers on the lower half of the side, males being numerous and often predominating. Pairs *in cop.* were common in the morning. Where the sides of marquees, closed during the night, were left standing by day more flies were found. Invariably, too, where the sides were rolled up into a corner, flies

were found within the roll. When the ground at the corner was cracked, flies were observed emerging from the earth and entering the folds of the roll, so that even after clearing such a roll in the forenoon one might later in the day take many flies from the same place.

(2) Earth cracks, in fact, proved to be effective day-shelters for *Phlebotomus*. The most important proved to be those occurring on the exposed surface of the soil where it had been dug out to make a level floor for tentage. The presence of flies in the cracks was easily demonstrated by blowing tobacco smoke or by squirting into the lower portion a little paraffin or even plain water. Within a tent twelve to fifteen flies might thus be driven from a crack less than a foot long and a few inches deep (August); when so expelled the flies generally came back after a short flight, and they had to be pretty thoroughly disturbed before they would travel any distance. Sometimes when driven from one crack they merely hopped along the cut surface and entered another.

(3) Sandbags employed to raise the level of the sides of tents, etc., are also a fertile source of trouble. The flies rest not only in the crevices between the sacks, but also enter the loose earth inside through the interstices of the coarse sacking. Where dug-outs are near wards, and their entrance is reinforced with sand bags, many flies will be found. In the tent which I occupied during my stay at Janes were several courses of sandbags. The following experiment was many times verified. When one opened up the tent and allowed strong light to fall so that one side of the person was illuminated and the other side in the shade, as one stood with the hand extended towards the sandbags, then though no flies had previously been noticed, bites began to be received in a very short time. After one or two such trials it was possible to see the sand-flies emerging from their retreat to the attack, which was always made on the shaded side. Besides the situation indicated, sand-flies shelter by day in hanging clothes, cupboards, blankets, beneath pillows, etc.

*Habits.* In studying the habits of *Phlebotomus*, much difficulty was at first experienced in keeping the flies alive. In test-tubes many died within two days, and few survived the third day. Not even when the air was kept humid could oviposition be induced.

In the end, suitable apparatus for handling the flies was improvised, and a list is appended.

Various devices for keeping the flies alive were tried during the first four weeks, and ultimately earthenware pots for single or smaller lots and cages for larger numbers proved most useful (see Appendix I). In the earthen pots—which were first suggested to me by Lieut.-Col. C. M. Wenyon—single specimens were easily kept and handled as follows. The fly was caught in a test-tube over the end of which a piece of cotton was loosely tied so as to form a small bag. The test-tube was then inserted through the sleeve of the cover. A little water was next poured into the pot and the cover tied on, the test-tube being fixed at any desired height above the water by a piece of string passed round the sleeve (fig. 4). Flies so kept showed an interesting periodic movement, staying all day in the cool, humid air below and appearing at the top of the tube during darkness. They were generally restless—particularly *papatasii*—about dusk. In cages provided with a tray filled with moist earth, stones, etc., a similar movement was observable, the flies hiding in crannies by day and emerging by night. A considerable proportion, however, remained by day crowded together in corners of the cage. They were frequently observed drinking from the wet earth.

In collecting sand-flies a pot was taken and covered and the flies introduced into the sleeve from the test-tube in which they had been caught. The sleeve was then tied and the pot stood in a bowl of water (fig. 5). To feed them it was necessary, first, to turn the flies loose for a time into a fly-proof box fitted with a sleeve to admit the arm (fig. 3). It was noticeable that flies kept in solitary confinement would seldom feed if the tubes were merely inverted over the arm.

In pots, unfed, a variable proportion of the flies—up to 50 per cent.—lived a week, 2 to 3 per cent. lived nine days, one ♀ (out of a batch of six hundred) lived exceptionally thirteen days. No ♂ was observed to live more than eight days under these conditions. All the females that lived nine days or over were examined as to the condition of their ovaries, and were found to be spent or practically so. In no instance could they be induced to feed in this state, though offered repeated opportunities—in the case of the last extending over the eleventh, twelfth and thirteenth days.



(1) *Biting, etc.* No males were detected in this act. The large number of this sex found with the females (as compared with *Culicidae*, where often the total catch from a ward will not include a single ♂) seems to be due to the fact that mating takes place after the female has had a blood feed. In all the couples examined the female had recently gorged herself. While feeding *Phlebotomus* is easily disturbed, the slightest movement of the skin being sufficient to put the fly to flight. It is thus difficult to study the process of biting in detail. The insect settles, pitches forward slightly, thrusting the somewhat stout rostrum downwards while the palpi (maxillary) diverge a little. I have not seen more than about one-third of the rostrum enter the skin, and the labium does not buckle up as in *Anopheles* when engaged in the same act. Only the labella are flattened out above and behind the piercing part of the associated organs. The wings are meanwhile poised ready for instant flight. Blood can be seen in the sucking stomach within sixty seconds. A full feed on an empty stomach occupies from four to four and a half minutes, at the end of which the fly suddenly withdraws the rostrum and makes off. For about forty-eight hours after a meal blood can be seen in the 'sucking stomach' over-lying the mid-gut (whose contents are brown or blackish) on the left side, and the whole gut may be cleared in five days, but the process generally takes longer. The females will feed at two-day intervals (possibly at shorter periods), taking less copious feeds, but attempts to find how many feeds could intervene before the completion of oviposition were abandoned. The digested blood is passed out ultimately in the form of small, dark, sticky drops; one or two specimens when captured had the stomach gorged with pale fluid and the gut hardly darker.

(2) *Movements.* In cracks and on rough earth *Phlebotomus* ordinarily proceeds by short runs varied by jumping to one side or another; on smooth walls or fabric more by jumping and short flights. Confined in a test-tube the males commonly mount in seeking an outlet. Females, if recently fed, on the other hand settle downwards, but after the gut has cleared and the eggs laid they behave much as do the males. When first introduced into a cage or confined space sand-flies make determined efforts to escape. They pass through astonishingly small openings, using the proboscis

apparently as a lever and emerging sometimes with a considerable proportion of the scales and hairs missing.

(3) *Drinking*. As *Phlebotomus* runs over the surface of a piece of earth it may sometimes be observed to plunge the rostrum into the earth. If there is moisture present the fly may remain for some time still. To observe what was happening, specimens were isolated in plaster of paris cells and watched under a Zeiss binocular. After they had been imprisoned for twelve hours the block was placed in clear water, which at once mounted to the floor and sides of the chamber containing the flies. Practically all the insects commenced to drink, some of them running about excitedly for an instant before settling. As was noted before, the tip of the rostrum (labrum, epipharynx and hypopharynx, etc.) was thrust distinctly into the plaster, and the labellae, flattened out, closely appressed to the porous surface. The short superiorly ensheathing maxillae generally moved backwards and forwards for a short time in front of the clypeus, and the maxillary palpi—rather widely divergent, nearly at right angles to one another—moved tremulously. But during drinking the mouth parts were still. Swelling of the abdomen could be traced as the drink proceeded. Flies, both sexes, were found to drink readily in this way about twice daily. They were tried with drops of fresh human blood on paper slipped into the cell, but without result. This can hardly be regarded as conclusive, as the blood dried rapidly. Defibrinated diluted blood was next allowed to soak into the cell containing flies, but did not prove specially attractive. The flies appeared to be able to extract only diluted serum—the corpuscular debris being retained by the plaster. In the same plaster cells cut deeper *Anopheles* was also induced to drink water.

### C. BREEDING AND REARING OF FLIES

(1) *Oviposition*. While the conditions in pots standing in water were congenial to the adult flies, there was apparently insufficient moisture within the pot to induce egg laying. Nor did females kept in a tube over moist paper oviposit. Complete success was, however, attained by the following methods. A thick 'tray' was partly filled with fragments of earth over which was lightly



sprinkled some crushed faeces of lizard, rabbit or man. The tray was then soaked in water and placed in a pot into which water had been poured to a depth of a quarter of an inch (fig. 5). The flies were then put in through the sleeve as usual, and the pot stood in water. After an interval the pot was opened; on washing out with as small a quantity of water as possible (20 c.c. was sufficient as a rule) a number of eggs were got from the sides. They were collected as follows. The liquid was centrifuged and the clear part used to rinse the pot again. This was done three times. Finally the sediment containing the ova was pipetted off and the ova themselves separated out under the microscope. The percentage of fertile eggs was extremely high, and their viability was not appreciably affected by their being for some time in water.

Besides the ova taken from the sides of the pot many were to be noted on the tray or among the earth, etc. To recover eggs so laid was an extremely tedious process. Some were picked up with a brush by direct observation, others recovered after carefully washing the contents of the trays. An improvement in gathering ova from the sides of the pot was effected by lining the inside with a single piece of cotton pressed down to fit exactly. The pot was then loaded as before. The eggs (which are at first pale and then darker like those of mosquitoes) showed up well against the cotton which was cut into strips and so passed below the microscope. A further advantage was that the cloth showed definitely the average position of the eggs laid on the sides of the pot to be along a band about three-quarters of an inch above the water surface (fig. 5, *o*.) This seems to indicate that there is a fairly precise optimum as regards moisture for oviposition. Eggs on the sides of the pot occurred singly, but the majority were found below the earth close to one another in bunches. The females had crawled into the smallest crevice possible and appeared to have projected the eggs still further. Beside all the larger collections of eggs in these cracks the mother could be found within two millimetres. She lay, as a rule, flattened between the surface of the tray and the overlying earth, with sometimes one or two eggs between the extremity of the abdomen and the rest of her laying. Sometimes an egg was seen attached to the terminal bristles of the genital appendages, and once or twice females had died with the egg between these appendages or blocking the genital

atrium. With a little care, it was easy to clear the earth from round each dead female and count the eggs laid. The larger batches contained from twenty-seven to thirty-four eggs. Sand-flies are not prolific insects, and the total ovarian content in *P. papatasii* runs from forty to fifty eggs. Assuming that these females had previously deposited one or two isolated ova on the sides of the pot, the proportion of eggs actually laid to the total possible is a high one. In some hundreds so secured there were practically none that failed to hatch. This high fertility and the large number of eggs laid may reasonably be held to indicate that natural conditions for oviposition had been secured.

Newstead, who in 1910 watched egg laying of *P. papatasii* in Malta, states (*a*) that the egg is projected some distance from the abdomen of the female; (*b*) that the process is so exhausting that the female may die after it; (*c*) that most of the eggs were laid *below* the moist blotting paper supplied to induce oviposition. With these notes the foregoing observations are quite in harmony, and it seems probable that the violent ejection of the ova serves to insert them into crannies where the abdomen of the mother cannot penetrate. Newstead saw the ova thrown about three times the length of the female abdomen, and this again agrees closely with the distance noted by myself.

In cages where the earth, etc., was placed on a shallow tray no excessive moisture ran up the sides (the water being completely absorbed), with the result that eggs occurred, so far as could be seen, only in pockets with dead females close by.

An attempt to determine whether the sand-flies in ovipositing showed any preference for one kind of faeces was inconclusive, owing to the development of microfungi, whose ramified mycelia enmeshed the eggs so that a complete count was impossible. The fungus growth was slight on lizard faeces either by themselves or mixed with earth, but very abundant on human faeces. So far as one could judge, however, plain earth or earth and lizard faeces held more eggs after some hundreds of females had been allowed to oviposit on plaster trays giving a choice of several kinds of larval food.

No female was noted to oviposit till four or five days after capture, and the eggs did not hatch till at least nine days more. The incubation period probably varies seasonally and specifically.

Ova belonging to at least two species were secured. Of these, one, the larger, was by its size, abundance, texture and resulting larva plainly *P. papatasii*. The other (probably *P. perniciosus*) was shorter and a little stouter. Apart from size, the two could easily be told towards the end of the period of incubation by two dark parallel lines which appeared shining through the shell laterally in each. In *papatasii* the lines at the caudal end of the ovum were rather broader and tapered towards the head. They could also be traced round the head and back towards the tail for a short distance. In the second species the calibre of the lines altered little between tail and head, and they extended nearly half-way back towards the tail on the other side. After hatching, these lines could be recognised as the caudal bristles, which are relatively short in *papatasii*. All through the first instar there is a kink in the caudal bristles, indefinite and near the extremity in *papatasii*, and much more decided and further back in the second species. The kink is, of course, at the point where the bristle is bent within the egg.

For the health and ultimate hatching of the egg a considerable degree of moisture is necessary—rather less, however, than is required to induce oviposition. Excess of moisture for a limited time has no effect on the viability of the eggs, for they may remain immersed a day or over and yet hatch. Eggs resting on earth surrounded by a thin film of water for three to four days also hatched. Drying, on the other hand, even a short time is fatal. A batch of eggs exposed in the shade overnight shrivelled before 11 a.m. next day, while in the daytime a few hours brought about the same result. (Time, 20th-25th September.)

(2) *Larvae*. When the egg is about to hatch the caudal bristles may be seen to move. There is a rippling movement also of the body from the tail towards the head on which, high up, almost vertical in position, is the well-developed dark egg tooth. Dehiscence of the shell is affected by a cut extending sometimes to half the length ventrally (?) and backwards for a short distance dorsally (?). The eyeless and legless maggot emerges slowly, and is at first entirely pale save for the egg tooth and bristles. The head, however, darkens in a few hours. In emerging the larva seems to be coming from two valves, but in some cases the line of

dehiscence is cut laterally as well as dorso-ventrally so that a relatively large oval piece of chitin falls from one side of the shell. Excessive moisture retards the process of hatching, always a slow one, and when the surface on which the egg rests has a thin covering film of water the larva may be found barely clear of the shell twenty-four hours after hatching began.

The newly hatched larva is sluggish, and, indeed, during the whole of this instar little activity is shown. It lies either flat on the supporting surface with the caudal bristles extended in the same line, or resting on the ventral caudal third to one-half of the abdomen, with the rest of the body raised and the last segment with its bristles slightly upturned. In this pose the larva is U-shaped. In a modification of this attitude the head is again thrown forward and the whole creature in profile S-shaped. The larva's progress is undulating. The head is first raised and the anterior segments stretched forward. The mandibles now press (or grip ?) firmly some inequality of the surface, and with this purchase the body is dragged slowly forward. Folds in the skin, and possibly the peculiar hairs of the body, aid in the process.

The tiny larvae begin to feed almost as soon as the head has darkened, *i.e.*, the mandibles are hardened. In feeding on lizard faeces they select the rough portion consisting of chitinous fragments mixed with partially digested fibre, and reject the more homogeneous limy part. Three individuals watched settled down respectively to the mandible of an ant, the head capsule of some small hymenopteron and the leg of a beetle, and ate the half-digested muscle fibre inside these structures. In the same way they enter and feed on the dead bodies of the parent flies. Older specimens of the first instar will also attack and devour larvae immediately after hatching.

Fungus was so troublesome (being rightly or wrongly blamed for the loss of many young larvae) that some substitute food was sought. Finely ground mixed blood and earth has proved satisfactory for this purpose. It is spread in lines on shallow plaster trays with the eggs near, and the whole covered with rough pieces of earth. The young larvae feed readily and appear to thrive, and no fungus has as yet developed.

Larvae in the first instar can survive excess of moisture for some



time. They sink readily in water, and are not sustained for any length of time by the surface film. When dried after lying twenty-four hours on wet soil they are lethargic but soon recover, yet like ova they are extremely sensitive to thorough drying and shrivel if exposed a few hours in the shade.

The first ovum to hatch did so on 20th September, and the first example in the second instar was seen on 26th September. In this stage the egg tooth has, of course, gone; the caudal bristles have increased from two to four, with a dark chitinous saddle connecting them. There is also a considerable increase in size.

In India, according to Howlett, there are separate broods of *P. papatasii* in August and September, while the wintering brood begins in late October or early November. At Janes I have seen no trace of the September brood, and the slow growth of these larvae hatched from late September eggs suggests that in Macedonia there may be one less brood than in India, and that the wintering brood commences here at least a month earlier.

Subsequent experience confirmed my expectation that the September hatched brood would hibernate. It was impossible to give much time to observing the larvae during the last week of September and the first fortnight of October, owing first to the congestion of the C.C.S. after the push, and later to the severe epidemic of influenza. But about the middle of October a considerable number of larvae, mostly in the second instar, were successfully taken to the Base and installed in the laboratory at 52 General Hospital. They were by this time very sluggish, and, in spite of being kept in moistened earth in a room which was heated at least during the daytime, latterly ceased to feed, while some died. One lot which had been left as it came from Janes was allowed inadvertently to dry up completely. On examining this tray in the third week of November (by which time it must have been quite dry for a month) I could find no larvae moving on the surface, but on breaking up the earth as a precautionary measure before throwing out the contents of the tray, I found several larvae in little pockets or blisters in the earth. They lay quite straight out but appeared stout and contracted, the integument wrinkled slightly towards head and tail, the gut empty or practically so. The only movement exhibited was a slight twitching from side to side of the extremities



when the animal was gently touched. By bringing larvae in this condition into moist, warm surroundings it was possible to revive them so that they recommenced to feed. Unfortunately the few experimented with were accidentally destroyed.

The bulk of my larvae had not revived naturally at the end of March, 1919, and the attempt to bring them to London failed. While it is not suggested here that *Phlebotomus* hibernates naturally under such conditions as have just been described (the opposite, indeed, is much more likely), it is evident that these larvae, once the critical stages of hatching and the first instar have been overcome, have unexpected powers of resisting dessication which must be of considerable help to the species in its natural breeding haunts in cracks, etc., where the conditions as regards moisture are variable.

#### D. PREVENTIVE MEASURES

(1) *Nets*. During my stay I used the sand-fly net issued to the troops with satisfactory results. With a flash-light at night one could see the flies settling on the net, yet none were noted to pass through. Once or twice sand flies (up to four) were found within the net in the morning, but these, I believe, came not from the outside but from blankets or below pillows, having got there during the process of bed-making. They also find harbour inside sleeping bags, and all bedding should be thoroughly shaken or beaten just before the net is adjusted for the night.

In this connection, however, it is worth recording that of thirty to forty mixed sand-flies placed in a small bag of the material from which the nets are made and hung up loosely, all escaped within half an hour. The flies behaved as they do when confined in a cage, pushing in all directions to find an outlet. I have found in the same way that a certain number of mosquitoes in a brood covered over with ordinary netting will manage to struggle through. The species in which this was noted were *Culex pipiens*, *Anopheles palestinensis* and *A. bifurcatus*. In spite of this the net in use appears to be efficient, though possibly where *P. minutus* preponderates a finer material might be necessary.

(2) *Repellents*. (a) Ordinary *Paraffin* is, if liberally applied, effective in keeping off the flies. It might be used as a stop-gap. M.T.C. drivers reported that they had employed it with good results, but I do not regard its regular use as advisable, and made no tests personally of the time for which an application remains effective. (b) *Mosquito Pomade* and (c) *Paraquit* were both tried. The former was issued to various members of the personnel of the C.C.S., who reported favourably on its use. Of the two, *Paraquit* is perhaps more pleasant to use. Well rubbed in, I found it effective for about three hours when one was sitting still or not moving actively. I found it sufficient to rub the *Paraquit* into the back of the hand, wrist and halfway up the arm towards the elbow, the neck, ears and round the scalp, but not into the hair. For the bites themselves I found appreciable relief by dabbing on a little rectified spirit with cotton wool.

(3) *General*. The breeding-sites of *Phlebotomus* hitherto recorded have been varied. Larvae have been found in soil along the sides of the embedded portion of the lower corners of old masonry; in rubbish in cellars, in earth cracks, etc.—the common feature in each case being darkness, moisture, and the presence of organic débris. While it is possible that at 31 C.C.S. the flies came in part from farm buildings near by, I think it most probable that the main breeding-places were in or round the hospital itself, in the cracks where the adults were themselves found. There was nothing in the distribution of these adults to suggest that their breeding-sites were other than generally distributed. Where there were more flies there were more men sleeping. In such circumstances prophylactic measures are difficult to carry out, and the only remedies available are probably palliative rather than radical.

The following suggestions are given for the treatment of tents and marquees. The floor should be levelled and cracks filled up with a mixture of *cresol* and *sand* or *sawdust*. *Clay* is unsuitable, as it is apt to crack in turn (this was repeatedly noted) and the most minute fissure will harbour many flies. The floor should then be liberally watered with a strong solution of *cresol*, and if possible covered with a ground sheet. Periodically, according to the severity of the plague, the tent should be closed and sprayed with a solution of formalin or fumigated with *cresol*. 1 per cent.

formalin has been recommended for spraying, but I believe this is too weak. In corrugated iron huts a 1 per cent. mixture of cresol in paraffin emulsion might usefully replace formalin for spraying. Around the tent all cracks (to a breadth of two feet from the tent) to be filled up and the soil to be sprinkled to the same width with cresol.

In latrines where crude cresol was experimentally sprinkled round the drums, sand-flies were reduced in number for two to three days, and ceased to bite on the exposed legs though bites were still received on the neck. A similar reduction was noted in a store where cresol was regularly sprinkled.

As far as possible, shallow soil with frequent outcrops of loose friable rock should be avoided in choosing a camp site. Loose soil removed in pitching tents should not be allowed to remain in camp. Building up with sandbags round tents, etc., is to be deprecated. Spilling of water or any liquid containing organic matter should be carefully avoided. The appearance of cracks in the soil should be watched for and counteracted by filling up when possible and spraying with cresol when the cracks are more extensive. From ten to fourteen days after the disappearance of the successive waves of sand-flies that occur from June to September a thorough sprinkling of cresol over suspected breeding-sites is to be recommended.

I have to thank heartily Lieut.-Col. Ievers, D.S.O., O.C., the 31st C.C.S., for his kindness in affording facilities for work during my stay at Janes, and very specially Lieut.-Col. C. M. Wenyon, C.M.G., who first suggested the investigation.

In conclusion, I should like to record my indebtedness to Captain Beer and Corporal Gibson, R.E. (143 Field Company) for carrying out my design for the breeding-cage (fig. 1), and to Captain Morrell, Dental Officer to the C.C.S., who supplied the plaster trays and cells.

## APPENDIX I

APPARATUS USED IN STUDYING THE LIFE HISTORY OF *Phlebotomus* spp.

CAGES may be made to suit requirements. (a) The following dimensions housed comfortably 600 sand-flies, 13 in. broad  $\times$  14 in. high  $\times$  6 in. deep, made of 1 in. wood, back of tin driven into the wood and nailed, on inside faced with cotton glued down and whitewashed. All joints glued and rabbeted. One or two panels of cotton fixed closely by strips of wood to afford ventilation. Tin tray to hold water in bottom. Flies admitted by tightly-corked circular opening at side. Front glazed to depth of about 10 in.—the glass set in putty. Narrow panel door below glass 12 in.  $\times$  2 in., made fly-proof by cloth edging. All round the door the joint is rabbeted and felt lined. The door may be

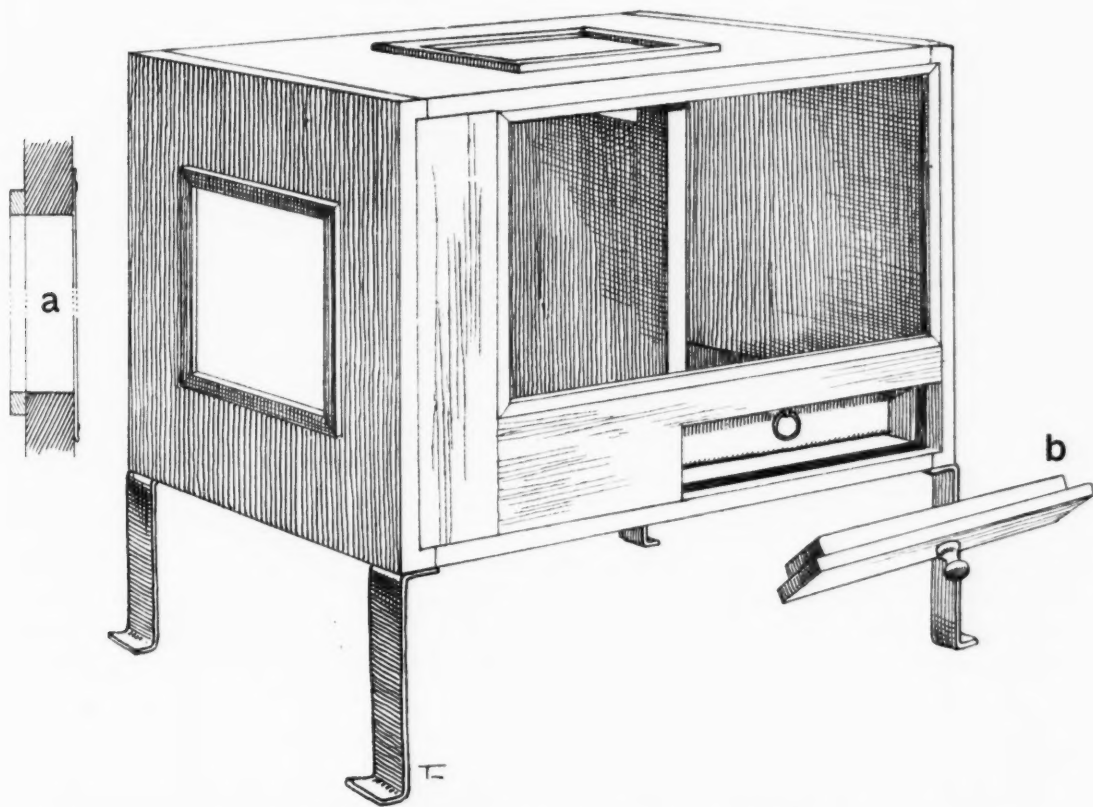


FIG. 1. Large breeding and feeding cage (11½ in.  $\times$  15½ in.  $\times$  14½ in.) front view. Glazed in front. *b*—Rabbit-fitting door giving access to tray for disposition of plaster receptacles of earth, etc., on which flies oviposit. The mid-partition cut down to give access of flies to host. *a*—Detail of ventilation showing the inner screen of stout wire-gauze and the outer cotton cloth.

secured by a clip. The middle surface of the rabbit pressing against the felt makes the joint fly-proof. (*b*) The above cage (no fig. given) is suitable when ova and larvae only are wanted. When the adult flies have to be fed for some time in numbers a more elaborate cage is required (figs. 1 and 2). It consists of two chambers. In the right-hand one the flies oviposit on material in tray. In the left chamber a rabbit or other small mammal can be accommodated for a time, but should not remain continuously with the flies. The animal sits on a wire grating and its urine and faeces are caught in a tray. Ventilation at top and side. In the latter



case the ventilator is double—cotton outside and strong wire-gauze towards the rabbit. Inside the door and fitted to the sides is a large sleeve through which the animal is introduced to the cage. In the middle the sleeve is held closed by an elastic belt to prevent the egress of the flies. The rabbit is introduced by forcing its head through the confined middle of the sleeve and sliding back the band over its body. The flies are put in as in cage (a) and gain access to their victim over and below the mid-partition, which is cut back for this purpose.

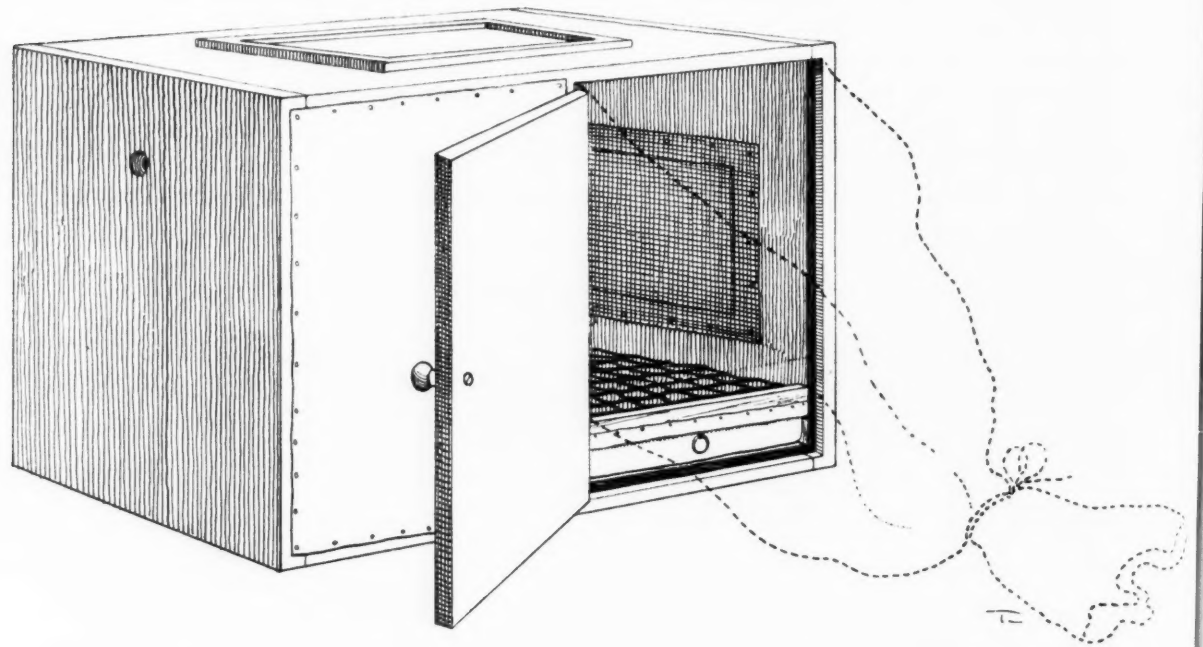


FIG. 2. The same from behind, showing the grid on which the rabbit is accommodated. Below, next to the door, is a wooden bar with a felt extension to make the faeces and urine tray fly-proof. The space above the grid is sleeved.

**FEEDING CAGE.** A quinine tin ( $10\frac{1}{2}$  in.  $\times$   $4\frac{1}{2}$  in.  $\times$   $6\frac{5}{8}$  in.) with strong lever lid (fig. 3) was used for this purpose; the lid was cut out leaving only the rim and the reflexed edge. A row of holes about  $\frac{1}{8}$  in. apart was drilled along the latter and a sleeve sewn tightly on; a side was next cut away to  $\frac{1}{4}$  in. from edge

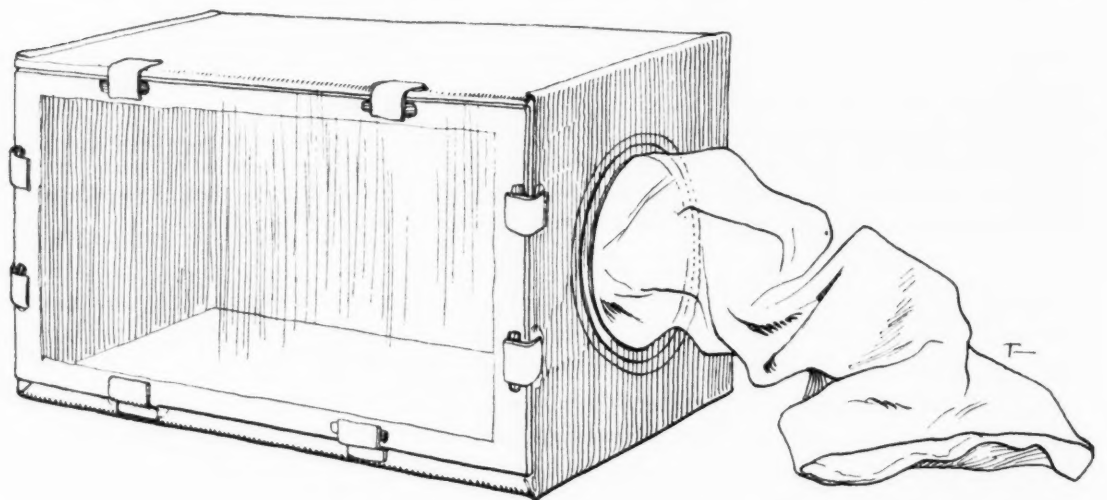


FIG. 3. Feeding cage with detachable sleeve.



and eight small clamps were soldered on, two at each side. The front was now glazed and puttied, and in the space between glass and clamps wedges of softish wood were fixed to prevent displacement of the glass. The inside of the cage was whitewashed. Flies kept in pots were released from their test-tube into the cage, fed, and easily recaptured.

The fact that no exact information was available in Macedonia as to the methods and apparatus used by previous workers was at first a considerable handicap. While it was easy to improvise ways of handling the flies in small numbers, many attempts were made before a satisfactory cage for dealing with large numbers of *Phlebotomus* was evolved. From the first cage made, 250-300 flies escaped in twelve hours, and only the closest search revealed the tiny escape hole. Some of the first boxes were constructed with grooved joints **U**, and the flies actually squeezed themselves round the **U** and so escaped. In other cases the flies disappeared gradually from the cage, but were not observed to escape. On taking the box to pieces they were found dead, packed solidly in the joints. Afterwards glued joints were tried with success. The superfluous glue must be carefully scraped off and the joint dusted e.g. with dry plaster of Paris to prevent the flies sticking. Glass jars are not suitable for housing *Phlebotomus* for any time, as the insects are liable to get stuck down by their wings.

In loading a pot with flies, the covers and tray are arranged as in fig. 5. The test-tube is then introduced into the sleeve perpendicularly, when the ♀♀ will generally drop down without trouble. The tube still in position is now laid

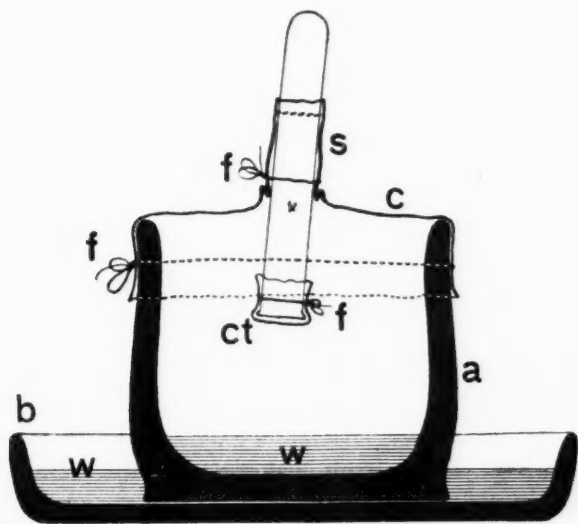


FIG. 4. Method of confining single flies required for observation. *a*—pot; *b*—outer tray; *w*—water; *c*—outer cover of pot with *s*—sleeve; *ct*—cover of test-tube; *f*—fastenings.

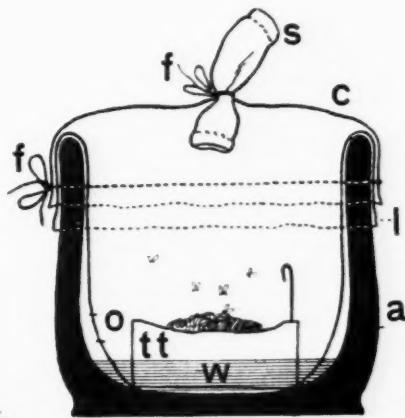


FIG. 5. *a*, *w*, *c*, *s* and *f* as in fig. 4; *l*—cotton lining of pot; *tt*—thick tray with faeces, etc.; *o*—approximate position of the zone on which most of the eggs not laid on the bait occurred.

horizontally for a moment and rapidly raised again to the first position. The ♂♂ come out, fly upwards, and are caught by the inner projection of the sleeve when the tube may be withdrawn.

The pot cover in fig. 5 is shown loose to prevent confusion with the inner lining, but in practice it must be tightly stretched and firmly secured, otherwise the flies squeeze down as far as they can get between the cover and the lining and are apt to be killed when the pot is lifted.

#### CELLS and TRAYS of PLASTER OF PARIS (fig. 6, *a-d*).

**CELLS.** These were cast a little wider at the base than at the top. In shape square. Measurements at base  $1\frac{3}{4}$  in., at top  $1\frac{1}{2}$  in., depth  $\frac{3}{4}$  in. The corners and angles were bevelled off. On the upper surface a square ( $\frac{1}{2}$  in. side) was cut to a depth of  $\frac{8}{16}$  in., over this was laid a No. 1  $\frac{3}{4}$  in. cover-glass, and the surface gently scraped away round the central hole till the cover-glass slid easily backwards and forwards over the cavity.

**TRAYS.** (*a*) *Thin* for cages, oblong, 3 in.  $\times$   $2\frac{1}{2}$  in.  $\times$   $\frac{3}{8}$  in., with a central hollow about  $1\frac{3}{4}$  in. in diameter sunk to about half the depth of the tray. (*b*) *Thick* for pots, circular, 2 in. diameter and  $\frac{3}{4}$  in.— $\frac{7}{8}$  in. deep. At one side a small length of wire, bent to form a handle, was let into the mould to facilitate lifting the tray from the pot. The central hollow of these thick trays was made by a 2 in. watch glass.

**EARTHENWARE POTS** of local manufacture, rough unglazed, circular,  $3\frac{3}{4}$  in.—4 in. in diameter and about the same height, with an inside depth of 3 in.; average thickness of walls  $\frac{1}{4}$  in. (see fig. 7, *a, b*). Covers for the above (fig. 7, *c*) were made of light, but closely-woven cotton cloth. In the centre was sewn a short sleeve of the same material. The sleeve was about  $1\frac{1}{2}$  in. broad by  $2\frac{1}{2}$  in. long above. It is an improvement to have it project below about  $\frac{1}{2}$  in. to prevent the ready egress of the flies after they have been introduced into the pot.

Wooden cages are loaded through the circular aperture at the side, which can be kept closed by a cork. Flies, if thirsty, generally go easily into observation cells, but sometimes ♂♂ can be induced to enter only by inverting the cell above the mouth of the tube and catching the ascending *Phlebotomus*.

The thin, flat trays are useful for isolating any given set of ova or larvae which it is desirable to watch. Their containers should have some water.

All cages and pots should be insulated (e.g., by water) to prevent depredations by ants, etc.

The sketches have been made by Mr. Terzi from apparatus brought back by the author.

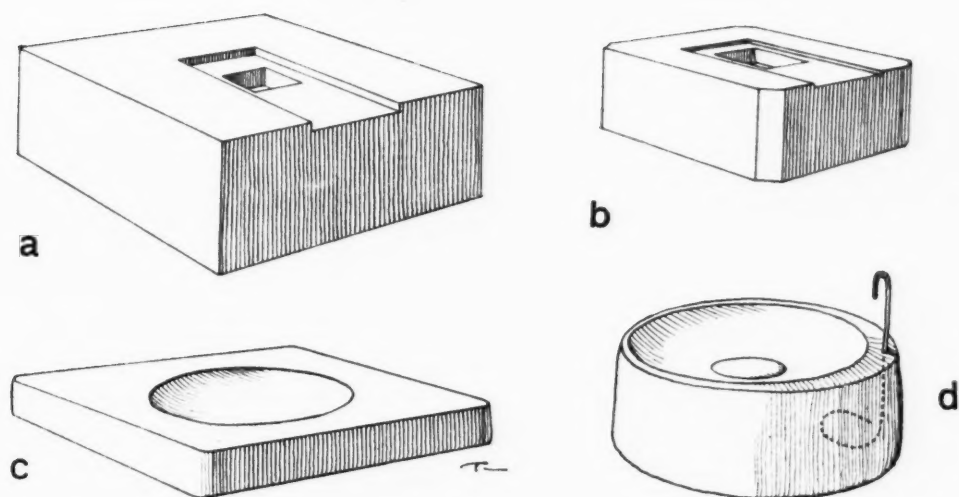


FIG. 6. *a*—large cell to demonstrate drinking habit of *Phlebotomus*, *b*—smaller block with larger well in which isolated ova were kept and development from day to day noted; *d* and *c*—thick and thin trays.

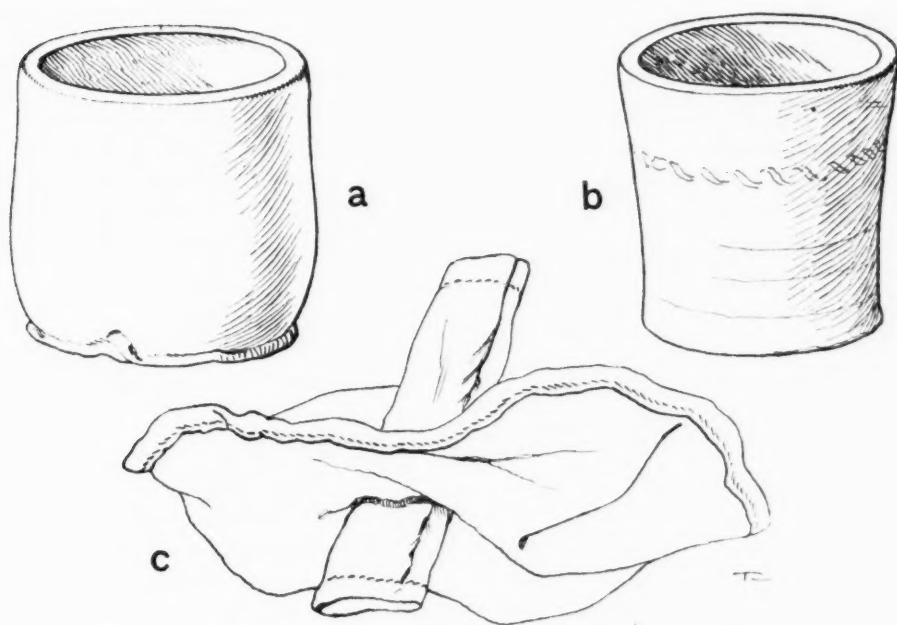


FIG. 7. *a* and *b*, Earthenware pots. *c*, Cover with sleeve.

## APPENDIX II

FEEDING OF THE LARVAE OF *Phlebotomus*

FAECES. Three kinds were used. To avoid any unpleasantness they were well dried and powdered before use, except in the case of the lizards' excreta, which were not offensive.

(a) *Lizards* (caught in some cases in cracks tenanted by sand-flies) were kept in a cage whose front and bottom were of coarse wire-gauze. The cage stood over a tray in which the faeces collected. The lizards were fed at first from fly-traps and on various insects specially caught for them. Latterly it was found that they would take grasshoppers readily, and as these *Orthoptera* made a more quickly satisfying meal and were easily secured, this became the staple ration for the lizards. The faeces were stored in test-tubes and did not generally develop fungus. Only the portion first passed, consisting of fragments of the exo-skeleton of insects to which some of the undigested internal parts still adhered, was of use. The more homogeneous urates—a limy-looking mass—were rejected.

(b) *Rabbit faeces*, well dried and crushed roughly.

(c) *Human faeces*, dried for twelve hours in oven and reduced to a very fine powder in a mortar.

PLAIN EARTH. Dried at air temperature; small stones removed; powdered in mortar. Small larvae were observed to ingest this, doubtless for the sake of the associated bacteria, etc.

EARTH AND BLOOD. Earth prepared as above. Human blood haemolysed, but not defibrinated. The two were mixed to the consistency of a thin mud. Next dried in oven into a cake, which was reduced again to a powder and stored for use. This was a most excellent food for young larvae. They gathered about the lines where it was laid, and its passage into their gut could quickly be traced under the binocular.

## NOTE ON TABLE (APPENDIX III)

From the middle of August to mid-September daily catches of *Phlebotomus* (up to 300 per day) were made at Janes. In all about 3,000 specimens were handled. Notes are given below on some of these lots so collected. Although I frequently watched *Phlebotomus* sucking blood, I do not think that a full feed was made on more than six or seven occasions, in all of which the times taken were very uniform, there having been not more than 15-20 seconds difference between any of them. A note on one such case is appended. No. 8.

No. of experiment	No. of flies	Larval food	Results of experiment	Surviving unfed
1	50	4.ix.18 Confined over lizard faeces	12.ix.18 All dead save one pair: ♂ moribund—died in two hours; ♀ active, refused to feed, though given choice of two hosts, died in 12 hours; ova laid in pot	26.ix.18 Larva (1) noted in 2nd instar ♂ 8 days. ♀ 8½ days
2	70	6.ix.18 Lizard faeces	16.ix.18 All dead. Ova laid, numerous, several batches, up to 34. Much mildew in faeces	21.x.18 Many larvae hatched. Two spp. present
3	100	7.ix.18 Broken rabbit faeces and earth	16.ix.18 All dead save one ♀; she will not feed; ova, several, enmeshed in mildew	21.x.18 Many larvae hatched. Two spp. present ♀ 11 days
4	120	8.ix.18 Human faeces on earth lump	18.ix.18 All dead, not many eggs	21.x.18 Many larvae hatched. Two spp. present
5	6-700	8-10.ix.18 Lizard, human, rabbit faeces in long tray	11.ix.18 Many dead	26.ix.18 Tiny larvae found completely enclosed in pockets formed by the breaking down of earth during moistening ♀ 8-10 days
6	100	Human faeces and earth	23.ix.18 All dead. Many eggs, but few on earth where mildew was dense; eggs in zone on cloth	
7	100	13.ix.18 Earth only	24.ix.18 Only one ♀ alive, will not feed; ova very numerous	26.ix.18 ♀ died, has not fed ♀ 13 days
8	1 ♀	28.ix.18 Specimen caught feeding. Gorged in 4 mins. 25 secs. Confined in test-tube in jar	30.ix.18 Sucking empty stomach half	7.x.18 Died REMARKS.—This specimen lived an hour or two over 9 days after feeding. Showed the usual night and day movements. Deposited faeces freely. 29.ix.—5.x.18

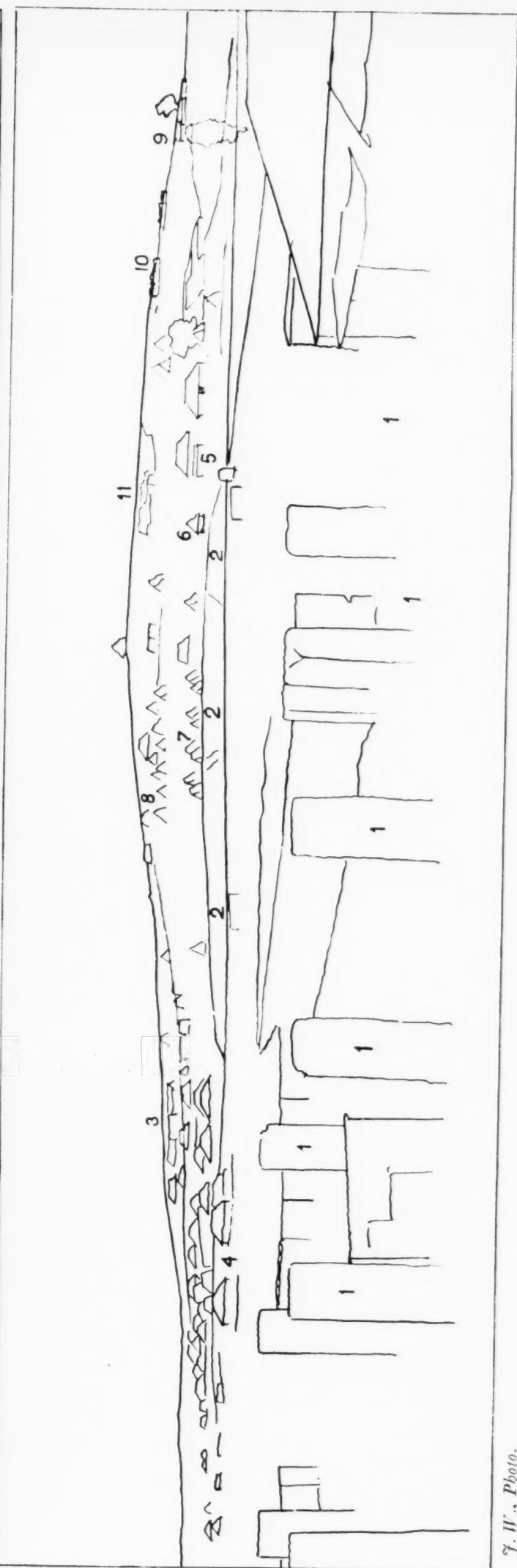


## EXPLANATION OF PLATE VIII

General View of Janes—31st Casualty Clearing Station,  
September, 1918.

1. Courtyard, pillars, etc., of Janes Farm.
2. Low, much cracked escarpment at roadside.
3. Hospital,—Wards, Sisters, etc.
4. Stores.
5. Pathological laboratory, Workshop, and Sanitary Section.
6. Entomological laboratory.
7. Personnel of C.C.S.
8. Officers.
9. Isolation wards.
10. Isolation wards,—Offices.
11. Personnel (N.C.O.'s).

The original centre of distribution may have been—1, where the flies were always numerous in cracks in the lower courses of the masonry. They were to be found also by day along 2; and men in 7 suffered severely. The largest catches were made at 9 and 10.



J. W., Photo.



# HUMAN INTESTINAL PROTOZOA IN AMAZONAS

BY

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*(Received for publication 6 March, 1922)*

The following findings of intestinal protozoa in five hundred persons were obtained from faeces collected for helminthic examination. The cases were unselected so far as their state of health was concerned, and were leading more or less normal lives, but almost all were infected with ankylostomes, and some showed malarial parasites in the blood. The majority of the stools were formed, a few were liquid, but no blood was observed in any.

The troops were living in barracks in Manáos, but were drawn from various parts of the State of Amazonas. The children examined were from the schools, and from a girls' orphanage in Manáos, the majority being girls. Matthews and Smith (1918 b) found little difference between boys and girls with regard to *E. histolytica*, *E. coli*, and *L. intestinalis* infections.

## METHODS

The faeces were collected in glass tubes having corks fitted with metal spoons. The tubes were distributed on one day and in the majority of cases collected during the following morning, instructions having been given that the specimen was to be taken on the morning of collection. Some specimens were not ready when due for collection and were collected later. All were examined within forty-eight hours of receipt.

One wet preparation in iodine (iodine 1, potass. iodide 2, water 100) was examined from each stool, but in cases where the diagnosis was in doubt further preparations were made.

One stool only was examined in each case so that only a fraction of the total infections are represented in the results.

The criteria employed in identifying the cysts were those described by Matthews (1918).

## RESULTS

The following protozoal cysts were found:—*Entamoeba histolytica* (Schaudinn), *Entamoeba coli* (Lösch), *Lambli* (*Giardia*) *intestinalis* (Lambl), *Chilomastix* (*Tetramitus*) *mesnili* (Wenyon), *Entamoeba nana* (Wenyon and O'Connor), and *Iodamoeba bütschlii* (Prowazek). *Trichomonas hominis* was not found in any of the five hundred cases, but three infectious were detected in diarrhoeic stools from hospital cases in Manáos.

The total figures are given in Table I.

TABLE I

						Federal Troops		Children of School Age	
						No.	Percentage	No.	Percentage
No. examined	...	...	...	...	...	251	...	249	...
<i>E. histolytica</i> .	Above 10 $\mu$	...	...	...	...	45	17.9	35	14.0
	Below 10 $\mu$	...	...	...	...	29	11.5	24	9.6
						69*	27.5	56†	22.5
<i>E. coli</i>	...	...	...	...	...	97	38.6	92	36.9
<i>L. intestinalis</i>	...	...	...	...	...	21	8.3	35	14.0
<i>C. mesnili</i>	...	...	...	...	...	11	4.4	4	2.8
<i>Blastocystis</i>	...	...	...	...	...	121	48.2	53	36.1
								In 144 cases	In 144 cases

\* 5 cases infected with both large and small cysts.

† 3 cases infected with both large and small cysts.

Figures for *E. nana* were not obtained as the structure of the nuclei of the cysts is not usually visible in iodine, and fixed preparations were not always made where its presence was suspected. It was, however, ascertained to occur.



Mixed infections are shown in Table II.

TABLE II.

								Troops	Children
No. examined	...	...	...	...	...	...	...	251	249
<i>E. histolytica</i> + <i>E. coli</i>	...	...	...	...	...	...	...	31	21
<i>E. histolytica</i> + <i>E. coli</i> + <i>L. intestinalis</i>	...	...	...	...	...	...	...	1	2
<i>E. histolytica</i> + <i>E. coli</i> + <i>C. mesnili</i>	...	...	...	...	...	...	...	3	2
<i>E. histolytica</i> + <i>L. intestinalis</i>	...	...	...	...	...	...	...	4	6
<i>E. histolytica</i> + <i>C. mesnili</i>	...	...	...	...	...	...	...	2	0
<i>E. coli</i> + <i>L. intestinalis</i>	...	...	...	...	...	...	...	6	10
<i>E. coli</i> + <i>C. mesnili</i>	...	...	...	...	...	...	...	6	2

#### SMALL CYSTS OF *E. HISTOLYTICA*

Small cysts (below  $10\mu$ ) of *E. histolytica* have been noted by James (1914), Woodcock and Penfold (1916), Wenyon and O'Connor (1917), Dobell and Jepps (1917), and others. A detailed account of them, and evidence of their differentiation from the larger cysts of *E. histolytica*, are given by Smith (1918 and 1919).

Most observers hold that these small cysts constitute a separate 'strain' of *E. histolytica*, but Woodcock and Penfold state that it is quite likely that this form is either a distinct species or distinct variety. Morphologically they are similar to the 'ordinary strain' of *E. histolytica*, except only in size, and it has been generally assumed that they belong to the same species. No work appears to have been published on their pathogenicity to animals. Dr. R. M. Gordon and I endeavoured to infect kittens with this small cyst without success, but as failure to infect controls with the large cyst also occurred, no conclusions could be drawn. As some doubt, therefore, exists regarding this so-called 'small strain' the findings of the two sizes have been recorded separately in Table I. In Table IV these figures are combined for comparison with the findings of other observers. Little difficulty was experienced in ascribing infections to their respective groups as very few cysts in the neighbourhood of  $10\mu$  were encountered. In eight cases cysts belonging to both 'strains' were present.

Table III has been compiled from a paper by Smith (1919) with the addition of the present series and shows the relative proportions of 'small' and 'ordinary strains' among the total *E. histolytica* infections. The figures for the two Manáos groups—troops and children—have been added together as the distribution of the two sizes is similar in each group. Attention is drawn by Smith to the small percentage of the 'small strain' in persons who had not been out of England.

TABLE III.  
Size of Cysts in *E. histolytica* Infections.

	England only	England and Abroad				Amazonas
	Matthews and Smith	Mackinnon	Mackinnon (1918)	Dobell and Jepps (1917)	Matthews and Smith	Present Series
	Non-dysenteric cases	Chronic dysenteric cases	Dysenteric and non-dysenteric cases	Dysenteric cases	Dysenteric cases	Troops and Children
Cases ... ..	98	56	209	200	306	125
Infections ... ..	99	59	225	215	325	133
' Ordinary ' % ... ..	85	64	47	65	66	60
' Small ' % ... ..	15	36	53	35	34	40

Table IV shows the findings of intestinal protozoa by various workers in different parts of the world. The figures represent percentages, and are all based on the results of one examination per case. The figures for *E. histolytica* include all 'strains' above and below  $10\mu$ , excepting those for Queensland where, Dr. Maplestone informs me, no cysts below  $10\mu$  were found. In the latter instance the stools were three to fourteen days old when examined.

Professor Kofoed has kindly supplied me with the figures for the United States of America. He states that they are probably higher than normal in the population as they contain large numbers of foreign immigrants and negroes from Florida. Figures published by Kofoed, Kornhauser and Plate (1919) for overseas troops of the United States Army are somewhat higher than those for home service troops.

	England				Malta	Egypt		Queensland	U.S.A.		Amazonas	
	Matthews and Smith					Wenyon and O'Connor			Kofoid	Home Service Troops	Children of School Age	Present Series
	(1918b)	(1918a)	(1918a)	(1919)		(1916)	(1920)					
	Children 0-12 years	Hospital Patients Adults and Children	Army Recruits	Male Asylum Patients 17-78 years	Bentham (1920)	Native Prisoners	Native Cooks	All Ages, 1-80 years				
No. examined	...	548	450	1098	207	200	524	87	500	576	249	251
<i>E. bistolytica</i>	...	1.8	1.5	5.6	9.7	27.5	13.7	11.5	4.6	3.9	22.5	27.5
<i>E. coli</i>	...	11.1	6.7	18.2	45.9	27.0*	48.6	20.7	26.4	14.1	36.9	38.6
<i>E. nana</i>	...	2.7	2.4	5.5	12.1	5.4*	0.0	0.0	0.0	29.3	+	+
<i>I. büschlii</i>	...	0.2	0.2	0.4	...	...	14.8	7.0	1.0		+	+
<i>L. intestinalis</i>	...	14.1	6.0	7.0	3.4	14.8*	0.6	7.0	11.8	6.4	14.0	8.3
<i>C. mesnili</i>	...	1.8	1.5	0.2	23.2	25.7*	0.2	1.1	2.2	2.2	2.8†	4.4
<i>T. hominis</i>	...	...	...	...	...	2.7*	0.0	1.1	...	0.2	0.0	0.0
<i>Blastocystis</i>	...	...	...	...	...	...	+	+	+	30.7	36.1†	48.2

\* In 74 cases.

† In 144 cases.

## SUMMARY

Five hundred persons living in Manáos were examined for intestinal protozoa with the results tabulated above.

The percentage of *E. histolytica* cysts recorded was somewhat higher than those reported from other countries for which figures are available, excepting Malta.

I am indebted to Dr. H. W. Thomas for allowing me to make use of the material collected for hookworm examination for this investigation.

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# A PARASITE RESEMBLING *PLASMODIUM FALCIPARUM* IN A CHIMPANZEE

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*(Received for publication 20 March, 1922)*

## PLATE IX

The following observations were made by us on a chimpanzee, *Anthropopithecus troglodytes*, at Freetown, Sierra Leone. The animal, according to the statement of the owner, had suffered from an attack of dysentery lasting from January 1st to January 16th, 1922. It was examined by us on the 11th and 12th of January, at which time it was passing blood, pus and mucus. The only organisms found in the faeces by microscopical examination were large numbers of *Blastocystis* resembling *Blastocystis hominis*; no bacteriological examination of the faeces was made; the cutaneous blood was examined, and no parasites were found. From January 16th to January 31st the animal was well. On January 31st and February 1st it refused food; its condition improved on the 2nd and 3rd, but the next day it became worse, and the owner handed it over to us. The chimpanzee was very thin, its hair was coming out and it was obviously ill; on February 5th it had an attack of diarrhoea; no blood was passed; *Strongyloides* larvae were present in large numbers in the faeces.

Malaria parasites were found in the blood on February 4th. They increased in number and the animal's condition became worse. On the 8th of February, in the afternoon, it was somnolent, and on the 9th it refused food and drink, lay motionless, was not easily



roused, and remained in any attitude in which it was placed without attempting to change it. At 8.30 a.m., after considerable retching, it vomited. As the animal's condition was grave and appeared to be associated with the increasing number of parasites in its blood, 5 grs. of quinine bisulphate in solution were administered orally at 10.30 a.m. From February 11th till 19th its condition improved; the number of parasites in the peripheral blood was reduced rapidly by the action of the quinine, but the blood was never free. On the 19th the blood was again heavily infected, more so than on any previous occasion; the animal was ill, but its condition was not as grave as it was on the 9th, and by the 21st its appetite returned and it began to recover without any quinine. On the 22nd the animal was lively and eating well, and the parasites in its peripheral blood were decreasing. On February 23rd, at 8.30 a.m., the animal appeared well and made a good meal. At noon the same day it was found lying in its cage in a condition of collapse and breathing with difficulty; it had vomited a large quantity of bile-stained material. Death occurred in half an hour.

*Post-mortem examination.* The immediate cause of death appeared to be innumerable small haemorrhages which were uniformly distributed over the whole surface of both lungs; these haemorrhages were very recent, and on examination proved to be caused by the presence of *Strongyloid* larvae. An account of the changes produced by the larvae and the sites in which they were found will be given in a future communication. The trachea contained a small quantity of regurgitated food, but this was not sufficient to cause obstruction. The vessels on the surface of the brain were dilated; there were no haemorrhages on the surface or in the substance of the brain; there was no meningitis. The spleen was not greatly enlarged; it was very dark in colour and somewhat harder than normal. The liver was dark and congested. The bone marrow was dark red. The kidneys and the heart appeared normal.

#### EXAMINATION OF SMEARS AND SECTIONS OF THE ORGANS

*Brain.* A few trophozoites and gametocytes were found; the capillaries were not blocked with parasites; pigment was present in small amount.

*Spleen.* Trophozoites and schizonts were found, but were not numerous; masses of pigment were present; there was considerable fibrosis.

*Liver.* This contained pigment in very large amount; it occurred in granules and in coarse masses; some of the smaller granules were found in the liver cells.

*Bone marrow.* Trophozoites and gametocytes were present, and coarse pigment was plentiful.

*Blood.* Trophozoites and gametocytes were present, but were not very numerous; very heavily pigmented leucocytes were common.

#### TYPES OF PARASITE FOUND IN THE BLOOD

1. Large amoeboid trophozoites resembling *P. vivax*, in pale enlarged red cells.

2. Large heavy looking trophozoites more or less band-shaped and equatorial, coarsely pigmented, resembling *P. malariae*.

3. Trophozoites resembling small rings of *P. falciparum*. The red corpuscles were not enlarged and retained their colour.

4. Gametocytes were found, indistinguishable from those of *P. falciparum*; they were never present in large numbers throughout the course of the disease.

No schizonts were found in the blood.

The *P. vivax* and *P. malariae* forms were scanty; they were found on the 4th and 5th of February, but were not seen subsequently. After the 5th of February the parasites seen were invariably of the *P. falciparum* type; they showed a certain amount of pleomorphism, but this was not more notable than in the case of the human parasite. The pleomorphism consisted in the appearance of slightly amoeboid and *tenue* forms. Crescents appeared in largest numbers on the 12th and 13th of February, but even then were not numerous; no exflagellating forms were found.

#### IDENTITY OF THE PARASITE

The few parasites of the *P. vivax* type corresponded to *P. inui*, Halberstaedter and Prowazek, 1907, in that the host cell was enlarged and pale, and did not present Schüffner's dots. As they and the *P. malariae* forms were not found in the blood on or after

the 6th February, the conclusions drawn from the experiments detailed below cannot be considered strictly applicable to them.

Reichenow (1920) records the discovery of parasites identical morphologically with *P. falciparum* in chimpanzees and gorillas. These parasites were always found by him in association with *P. vivax* and *P. malariae* forms. He concluded that anthropoid apes are as sure a source of danger to Europeans living in West Africa as are negroes.

The conclusion of Reichenow as to the identity of the parasite he found in gorillas and chimpanzees with the human parasite is interesting. The establishment of this identity would necessarily lead to important inferences. It would mean that anophelines which had fed on infected anthropoid apes could acquire salivary gland infection. Such anophelines, in parts remote from human habitation, would be capable of infecting any human being who came within their range. In this way they would constitute a permanent danger to persons employed in opening up new areas.

Reichenow's conclusion appears to us too far-reaching in view of the fact that it is based on morphological grounds only. If his conclusion is correct, it becomes difficult to understand why inoculations from human beings infected with malaria into chimpanzees should fail. The only successful inoculation of malaria from a human being into an animal is that performed by Mesnil and Roubaud (1920). These authors succeeded after several attempts in inducing a transient infection with *P. falciparum* in one of two chimpanzees. The incubation period was ten days, and the animal recovered spontaneously after another ten days. It is significant that in the two experiments using as vector *A. maculipennis* which had been infected from a case of *P. falciparum*, transmission to the chimpanzees failed entirely. This alone would suggest that *P. falciparum* is not easily transmissible to the chimpanzee, in view of the ease with which infective anophelines transmit *P. falciparum* to human beings in laboratory experiments. The failure of Mesnil and Roubaud to transmit malaria to chimpanzees by the bite of infected anophelines raises the question as to whether the *P. falciparum* forms observed by them in the chimpanzee were really due to the inoculation or were a relapse of the parasite which occurs naturally in the chimpanzee.

In order to determine whether the parasite resembling *P. falciparum* found by us in the chimpanzee was capable of infecting human beings, we performed the following experiments.

#### EXPERIMENTS WITH LABORATORY-BRED *A. COSTALIS*

Laboratory-bred *A. costalis* were allowed to feed on the chimpanzee on two successive nights. After a lapse of from four to fourteen days from the first feed, forty mosquitoes were dissected, and in no case was infection found either in the gut or salivary glands.

#### EXPERIMENTS WITH INJECTIONS OF INFECTED BLOOD

Two Europeans were given subcutaneous and intravenous injections of blood from the chimpanzee. The first subject had never had malaria, and had taken prophylactic doses of five grains of quinine bisulphate daily from January 10th to February 6th, 1922. The last dose was taken at 7 a.m. on February 6th. On February the 7th, at 5 p.m., he received subcutaneously 1 c.c. of the blood of the infected animal. An hour later slight nausea ensued, which lasted two hours. The local reaction was slight. On the 9th of February, at 10 a.m., the same subject received an injection of 0.4 c.c. of the animal's blood into his right median basilic vein. At this time the animal's infection was heavy, *i.e.*, four rings to the field (Obj. 1/12, Oc. 0, Leitz). Slight nausea followed a quarter of an hour after the injection, and lasted a few hours. The subject's blood was examined twice daily from the date of the first injection, but no parasites were found. During an observation period of twenty-eight days, no infection occurred. An interesting fact was observed, namely, that from the 12th to the 14th of February transient urticarial patches occurred, localised round the site of the first inoculation. These patches appeared and disappeared several times during the course of the day.

The second subject had previously suffered from malaria, and recently, within a year, from a *P. falciparum* infection, but had been free from relapse during the last six months. He was taking two grains of quinine bihydrochloride daily until the 7th February.



He received on February 19th, at 7 p.m., 1 c.c. of the animal's blood subcutaneously; at this time the animal's blood showed as many as nine rings to a field. No local or general reaction followed. On the 20th February, at 5 30 p.m., he received 0.2 c.c. of the animal's blood intravenously. Examination of the subject's blood before the first inoculation was negative, as were also subsequent examinations. No infection occurred during an observation period of seventeen days after the second inoculation.

The results of the above experiments lend themselves to two explanations, viz., that the parasite is *P. falciparum* which has lost its infectivity for man by passage through the chimpanzee, or that it belongs to a new species of the genus *Plasmodium*. In view of the limited number of experiments performed, we consider it premature at present to decide definitely between these two interpretations. Our experiments so far certainly do not confirm Reichenow's conclusion that chimpanzees as reservoirs of *P. falciparum* are a source of danger to Europeans in West Africa.

#### SUMMARY

A parasite morphologically indistinguishable from *P. falciparum* was found by us occurring naturally in a chimpanzee in Freetown, West Africa. This parasite appears to be the same as that described by Reichenow in chimpanzees and gorillas, and stated by him to be the human parasite.

Laboratory-bred *A. costalis* fed on this chimpanzee failed to become infected, but, as stated above, crescents were few and exflagellation was not observed.

We have failed to transmit the infection to two human subjects by subcutaneous and intravenous inoculation.

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## EXPLANATION OF PLATE IX

Forms of parasite found in the blood of the chimpanzee.

Fig. 1. *P. vivax*-like form.

Fig. 2. *P. malariae*-like form.

*P. falciparum*-like forms :—

Figs. 3-10. Small rings.

Figs. 11-14. Large rings.

Figs. 15-17. Amoeboid forms.

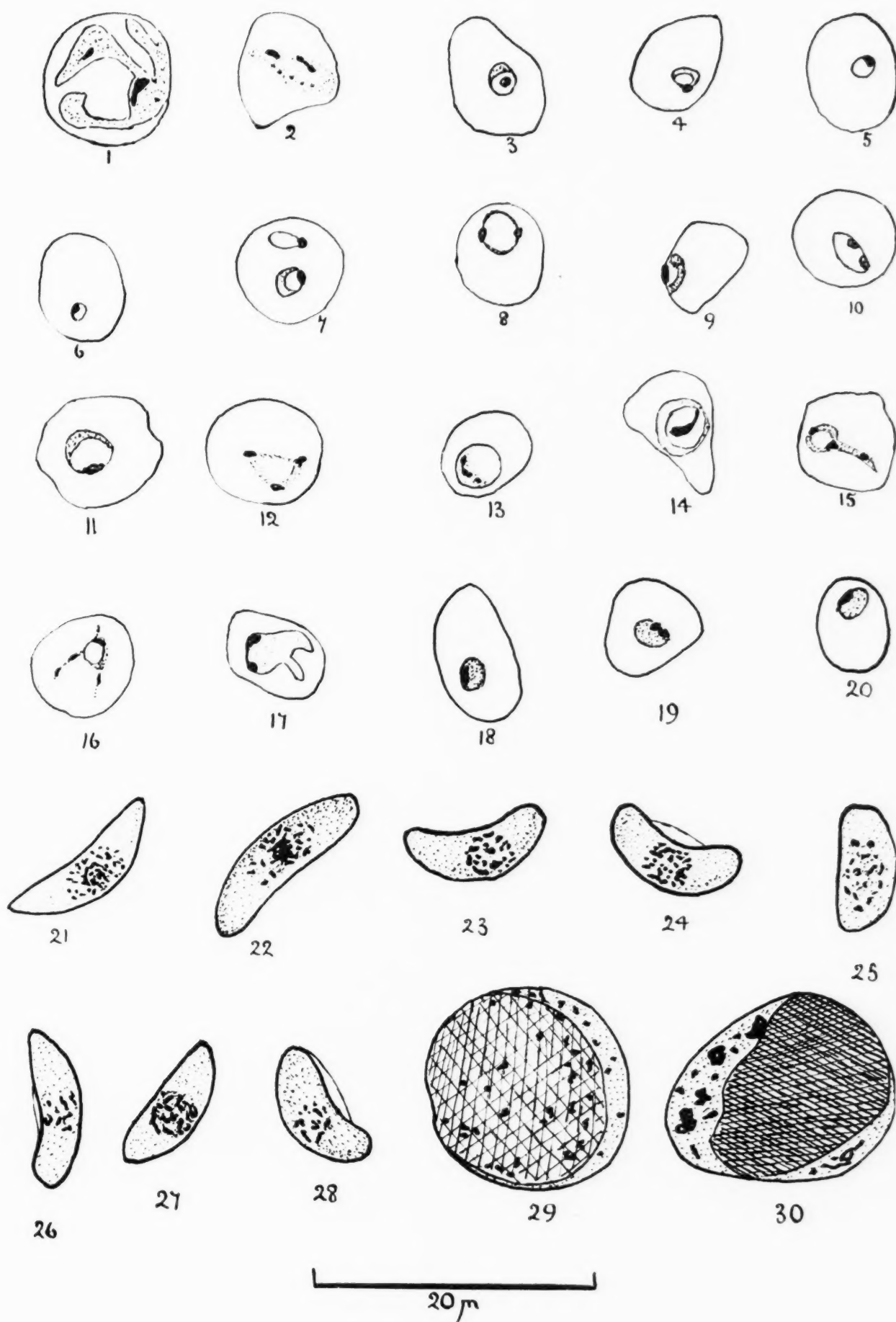
Figs. 18-20. Solid forms.

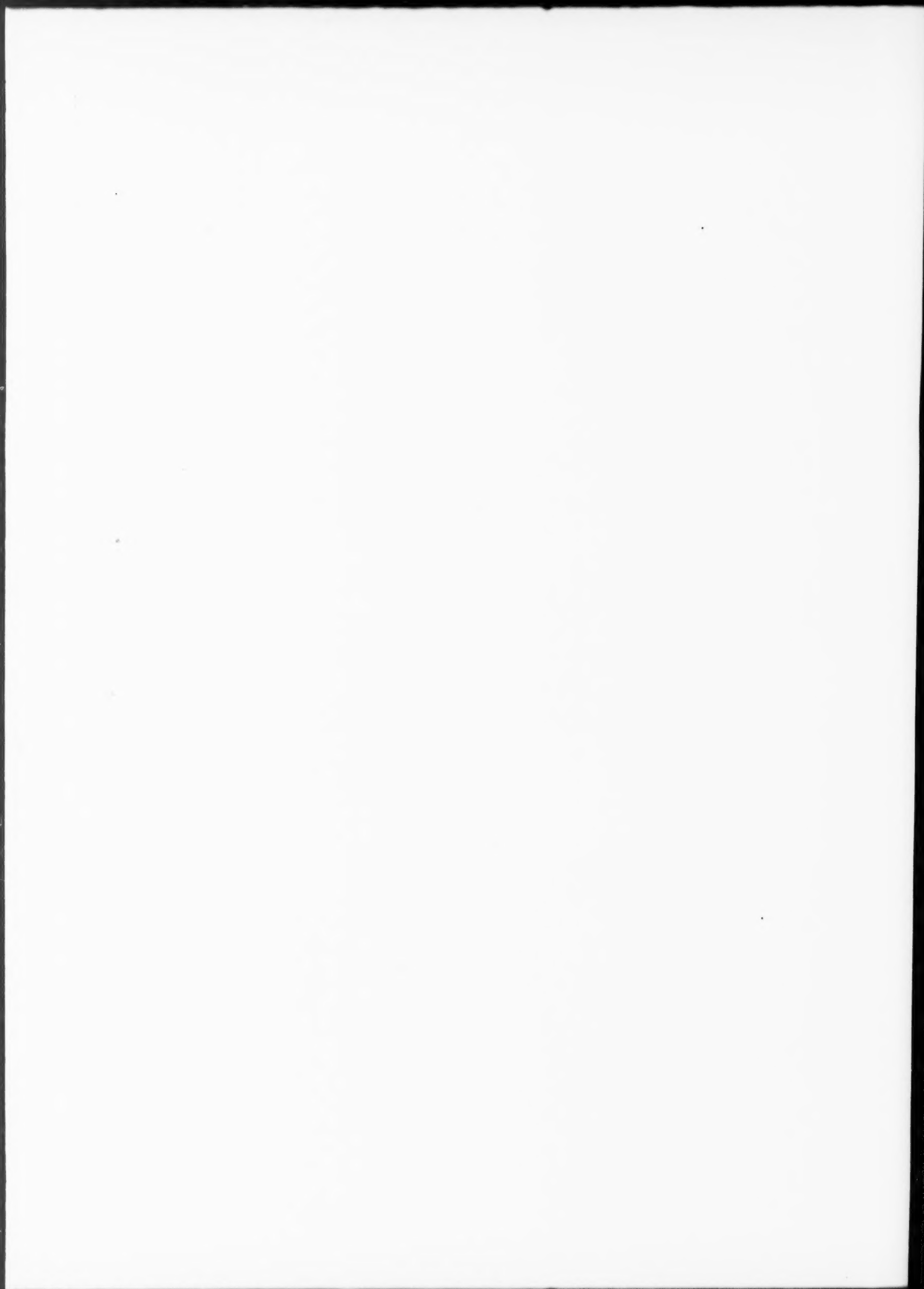
Figs. 21-28. Crescent forms.

Pigmented leucocytes :—

Fig. 29. Pale nucleus and finer pigment.

Fig. 30. Dark nucleus and coarse pigment masses.









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# THE SIGNS OF FILARIAL DISEASE

BY

B. BLACKLOCK

(Received for publication 20 March, 1922)

The signs of filarial disease are those signs which are held to have been produced by the previous pathological action of adult *Filaria bancrofti* and its embryos; so varied in their character are these signs and so diverse is their relationship to the presence of microfilaria in the cutaneous blood, that it is well to have some definite mode of tabulating them for purposes of comparison.

Stephens (1921) has established a standard which will prove of value in this direction, in his analysis of Manson-Bahr's (1912) Monograph on Filariasis. His tables I,\* II, XII and XIII deal with the percentage occurrence of microfilaria in the blood in persons in whom the presence of signs has been established; his tables III, IV, XIV and XV deal with the percentage occurrence of signs in persons in whose blood the presence of microfilaria has been established; his table V deals with the percentage occurrence of microfilaria in the blood and the percentage occurrence of signs at different age periods, and his table VI deals with the same facts in respect to the localities in which the patients were examined.

The results obtained by me while examining natives at Mabang, in the Protectorate of Sierra Leone, are set out below in such a way as to be comparable with certain of those tables. The investigation was carried out during the months July and August, 1921; the number of persons (240) dealt with in this paper is the residue obtained after excluding cases which presented other microfilaria in the blood or were not sufficiently examined from refusal to have blood films taken. The hours during which the examinations were made were from 6 a.m. to 6 p.m.; there was a considerable gang of

\* Professor Stephens has asked me to draw attention to the facts that in his Table I, 103 should read 108, and 19.5 should read 20.5; while in Table III, 269 should read 265, and 39.5 should read 38.7.

men who were employed, many of them in night-work on the river, cutting mangrove trees. This possibly contributed to the appearance of *Microfilaria bancrofti* in small numbers in the day time: the examination was confined to a stained thick film of each case. The notes of the occurrence of any pathological condition seen on examination were made first, and the blood films taken at the same time were examined afterwards. Before proceeding to the consideration of the tables, it is necessary to state that the figures in the tables are to a certain degree weighted on the side of 'signs of filarial disease.' For example, a diagnosis of elephantiasis was made to include not only cases such as are shown in the photograph (text-fig. 1), but also cases of oedema in which the hyperplasia



FIG. 1.

of the cutaneous structures was by no means an outstanding feature; also it includes very circumscribed conditions of hyperplasia with fissuring of the skin at the apex of the otherwise normal-looking scrotum. The influence of the length of the scrotum in producing such lesions is perhaps a factor deserving attention, in view of the frequency with which contact with the ground must occur in squatting down in the native fashion. In one case in which, however, there was no evidence of skin lesion, nor of any patho-

logical condition of the cord and testicles, the scrotum, on a foot-rule placed behind it against the perineal origin, measured ten inches. It is clear that a scrotum of much less dimensions than this will very frequently be in contact with the ground, and the risk of septic infection of the skin at the apex has to be considered. Again, a diagnosis of enlarged lymphatic glands was made to include two categories, one comprising fifty-eight cases in which the glands were visibly enlarged and another comprising seventy cases in which the glands were not visibly enlarged, but were considered on palpation to be enlarged. It is possible unconsciously to weight the 'signs' if the blood films are first examined and the micro-filaria-in-the-blood cases are examined subsequently for signs; the process is to a great degree governed by the pre-disposition of the individual in favour of what he may consider the signs properly to be associated with the existence, past or present, of filaria adults as shown by the presence of microfilaria in the blood. The risk of weighting the microfilaria is less owing to the fact that the observer usually has his own definite method of making his blood preparations; consequently, if he fails to find microfilaria in the usual sample taken from a case in which he has found signs, he could not take another larger sample in order to discover them without conscious effort. It would appear preferable, therefore, to make the notes of signs first and the examination of blood films afterwards.

TABLE I.

Showing percentage infected with microfilaria among those with and without signs of filarial disease\* at Mabang.

	Number examined	Number infected with microfilaria	Percentage
With signs of filarial disease ... ..	138	29	21.0
Without signs of filarial disease ... ..	102	18	17.6

\* The 'signs of filarial disease' selected for these tables are those included in Stephens' tables, i.e., enlarged glands, hydrocele, enlarged testis, abscess and elephantiasis.

CONCLUSION. Microfilaria is commoner among those with signs of filarial disease than among those without signs of filarial disease.



TABLE II.

Showing percentage infected with microfilaria among those with and without particular signs of filarial disease at Mabang.

	Number examined	Number infected with microfilaria	Percentage
With elephantiasis ... ..	11	2	18.2
Without elephantiasis ... ..	229	45	19.6
With enlarged glands ... ..	128	29	22.6
Without enlarged glands ... ..	112	18	16.1
With hydrocele ... ..	9*	1	11.1
Without hydrocele ... ..	176*	44	25.0
With enlarged testis ... ..	7*	1	14.3
Without enlarged testis ... ..	178*	44	24.7
With abscess ... ..	8	0	0
Without abscess ... ..	232	47	20.2

\* Males.

CONCLUSION. Microfilaria is commoner among those with enlarged glands than among those without enlarged glands; microfilaria is less common among those with elephantiasis, hydrocele, enlarged testis and abscess than among those without these signs of filarial disease.

The effect of considering as signs of filarial disease only those glands which are visibly enlarged is as follows.



### III

TABLE IIa.

Showing percentage infected with microfilaria among those with and without visibly enlarged glands at Mabang.

	Number examined	Number infected with microfilaria	Percentage
With visible glands ... ..	58	11	19'0
With no visible glands ... ..	182	36	19'7

CONCLUSION. Microfilaria is less common among those with enlarged glands than among those without this sign of filarial disease.

In addition to the above lesions, notes are available of conditions not usually considered to be associated with filarial disease, namely, hernia and ulcers.

TABLE IIb.

Showing percentage infected with microfilaria among those with and without hernia and ulcers at Mabang.

	Number examined	Number infected with microfilaria	Percentage
With hernia ... ..	15	3	20'0
Without hernia ... ..	225	44	19'5
With ulcers of skin ... ..	18	2	11'1
Without ulcers of skin ... ..	222	45	20'3

CONCLUSION. Microfilaria is commoner among those with hernia than in those without hernia, but is less common among those with ulcers than among those without ulcers.

TABLE III.

Showing percentage exhibiting signs of filarial disease among those with and without microfilaria at Mabang.

	Number examined	Number with signs of filarial disease	Percentage
With microfilaria ... ..	47	29	61.7
Without microfilaria ... ..	193	109	56.5

CONCLUSION. Signs of filarial disease are commoner among those infected with microfilaria than among those not infected with microfilaria.

TABLE IV.

Showing percentage exhibiting particular signs of filarial disease among those with and without microfilaria at Mabang.

	Number examined	Number with particular signs of filarial disease	Percentage
With microfilaria ... ..	47	ELEPHANTIASIS 2	4.3
Without microfilaria ... ..	193	9	4.7
With microfilaria ... ..	47	GLANDS 29	61.7
Without microfilaria ... ..	193	99	51.2
With microfilaria ... ..	45	HYDROCELE 1	2.2
Without microfilaria ... ..	195	8	4.1
With microfilaria ... ..	45	ENLARGED TESTIS 1	2.2
Without microfilaria ... ..	195	6	3.1
With microfilaria ... ..	47	ABSCESS 0	0
Without microfilaria ... ..	193	8	4.1

CONCLUSION. Cases of enlarged glands are commoner among those infected with microfilaria than among those not infected with microfilaria: cases of elephantiasis, hydrocele, enlarged testis and abscess are less common among cases infected with microfilaria than in those not infected with microfilaria.

TABLE IVA.

Showing percentage exhibiting visibly enlarged glands among those with and without microfilaria at Mabang.

	Number examined	Number with particular signs of filarial disease	Percentage
With microfilaria ... ..	47	VISIBLE GLANDS 11	23.4
Without microfilaria ... ..	193	47	24.3

CONCLUSION. Visibly enlarged glands are less common among those infected with microfilaria than among those not infected with microfilaria.

TABLE IVB.

Showing percentage exhibiting hernia and ulcers of the skin among those with and without microfilaria at Mabang.

	Number examined	Number with particular signs of filarial disease	Percentage
With microfilaria ... ..	47	HERNIA 3	6.4
Without microfilaria ... ..	193	12	6.2
With microfilaria ... ..	47	ULCERS OF SKIN 2	4.2
Without microfilaria ... ..	193	16	8.3

CONCLUSION. Hernia is commoner among those infected with microfilaria than among those not infected with microfilaria: ulcers are less common among those infected with microfilaria than among those not infected with microfilaria.

TABLE V.

Showing percentage infected with microfilaria and percentage showing signs of disease at various age periods in the population examined at Mabang.

Age period	Number examined	Percentage infected with microfilaria	Percentage showing signs of filarial disease	
		MALES		
1-10 ... ..	7	0	42.8	42.8*
11-20 ... ..	48	12.5	58.3	54.2*
21-30 ... ..	93	36.5	75.3	67.7*
31-40 ... ..	24	16.6	62.5	45.8*
41-50 ... ..	11	0	63.6	45.4*
51-60 ... ..	2	50.0	...	...
		FEMALES		
1-10 ... ..	11	0	36.4	...
11-20 ... ..	9	11.1	11.1	...
21-30 ... ..	16	0	25.0	...
31-40 ... ..	9	0	22.2	...
41-50 ... ..	10	10.0	40.0	...
51-60 ... ..	0	0	...	...

\* Hydrocele and enlarged testis excluded.

CONCLUSION. The results, so far as they can be considered from such small numbers in some groups, do not agree closely with those in Stephens' Table V, which is produced here for comparison with the above and for other reasons.

TABLE VI.

Showing percentage infected with microfilaria and percentage showing signs of disease at various age periods in the population examined in Fiji.

Age period	Number examined	Percentage infected with microfilaria	Percentage showing signs of filarial disease	
		MALES		
1-10 ... ..	85	1.2	18.8	18.8*
11-20 ... ..	117	21.4	59.0	59.0*
21-30 ... ..	108	39.8	64.8	64.8*
31-40 ... ..	83	47.0	78.3	78.3*
41-50 ... ..	63	52.4	69.8	66.6*
51-60 ... ..	35	37.1	71.4	62.8*
61- ... ..	21	38.1	76.2	61.9*
		FEMALES		
1-10 ... ..	66	10.6	9.1	...
11-20 ... ..	108	24.0	20.3	...
21-30 ... ..	124	22.6	31.4	...
31-40 ... ..	61	22.9	34.4	...
41-50 ... ..	41	34.1	26.8	...
51-60 ... ..	19	26.3	52.6	...
61- ... ..	13	30.7	23.0	...

\* Hydrocele and enlarged testis excluded.

CONCLUSION. No close relationship between the two sets of percentages is evident.

There are in the above table several figures which appear to me to be of great interest. It will be observed that in the males of the age period 1 to 10 there are 1.2 per cent. infected with microfilaria; total examined eighty-five. In females, however, in the same age period, there are 10.6 per cent. infected with microfilaria; total examined sixty-six. Again the percentages of males infected in periods 1 to 10 and 31 to 40 are in the ratio 1 : 39.2, while the percentages of females infected with microfilaria in the same age



periods are in the ratio 1 : 2.2. These figures appear somewhat anomalous, but not more so than the following. At the age period 1 to 10 in males, while 1.2 per cent. are infected with microfilaria, 18.8 per cent. show signs of filarial disease; total examined eighty-five.

Let us suppose for a moment that pre-existing infection with *Filaria bancrofti* can be detected by two means:—

- (1) Presence of microfilaria in the blood.
- (2) Presence of signs of filarial disease.

If one refers to the figures above, one observes that in the 1 to 10 age period in males we find 15.7 times as many sign cases as microfilaria cases. That is, roughly, of sixteen boys of 1 to 10 years of age infected with this filaria, fifteen are diagnosable by signs, while only one is diagnosable by blood examination. This would be a very remarkable fact, and would have been worthy of attention being drawn to it, had it not been difficult to understand why girls of the same age period gave a totally different ratio, the sign cases in girls of 1 to 10 years being only 9.1 per cent., while the microfilaria cases are 10.6 per cent.; sixty-six examined.

One explanation which can be suggested is that in boys the signs of filarial disease come into evidence without or before the appearance of microfilaria in the blood, while in girls of the same age the signs of filarial disease are accompanied by the presence of microfilaria in the blood. How to explain the facts, if we accept this hypothesis, is the next question; the early prominence of signs of filarial disease in boys might be attributed to the fact that in the figures for boys there are included the signs which appear under the designation hydrocele and enlarged testis, which signs are not represented in the figures for girls. It is possible to suppose that filaria attack the male genitals early in life and that two results arise from this, the first positive, *i.e.*, the production of lesions, hydrocele and enlarged testis; the second negative, *i.e.*, the failure of the adult to produce microfilaria in the blood. A glance at the extension column shows us that this explanation does not suffice; there we see that even excluding the male genital signs, namely hydrocele and enlarged testis, the figure representing signs of filarial disease in males 1 to 10 remains the same. In fact, it appears that signs of filarial disease affecting the male genitals do not even exist in this age period.

The absence of any standard by which to judge what may properly be considered as 'signs of filarial disease' may be the reason for the anomalies referred to; it appears probable that 'signs of filarial disease' is a term which requires a further and critical examination and modification. Although enlargement of the spleen may reasonably be called a sign of malarial disease, it would often be erroneous to diagnose malaria simply from enlargement of the spleen. To diagnose filarial disease from the discovery of enlarged glands, hydrocele, and so on, may be no less misleading.

#### SUMMARY

1. Two hundred and forty cases were examined at Mabang, Protectorate of Sierra Leone, in July-August, 1921, with a view to establishing a correlation between 'signs of filarial disease' and the occurrence of *Microfilaria bancrofti* in the blood.
2. The figures obtained do not show the same kind of correlation in these respects as do Stephens' figures, obtained by analysing Manson-Bahr's Fiji cases.
3. The figures obtained show that in this series many of the 'signs of filarial disease' have no more correlation with the presence of microfilaria in the blood than has hernia; some have less.



## TWO FURTHER CASES OF CARDIAC ANEURYSM

BY

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AND

J. W. S. MACFIE

*(Received for publication 3 April, 1922)*

### PLATE X

The two following cases of cardiac aneurysm recently examined at Accra in the Gold Coast, West Africa, the one of the left auricle and the other of the anterior cusp of the mitral valve, are briefly recorded on account of their comparative rarity, and because they form a somewhat interesting series with the cases previously described by us (1920).

CASE I. A European man, aged about thirty-two years, who died suddenly on the beach at Accra when waiting to be conveyed on board the homeward-bound mail steamer. During the latter part of the war, the deceased served in the Flying Corps and lost his right arm as the result of an accident, the arm having been injured by coming in contact with a moving propeller. Previous to this he had been employed by a firm of contractors in the Gold Coast, and at the end of the war he returned to this Colony as a foreman in the Public Works Department. At the time of his death he had just concluded a year's tour of service spent at Accra, or in the neighbourhood of that town.

We are indebted to Dr. C. V. LeFanu for the following clinical notes of the case. Five months before his death the patient had a slight attack of malaria, but with this exception had not reported 'sick' during his tour. A week before he should have sailed for England he was examined for his Final Medical Certificate. Upon auscultation of his heart a peculiar bruit was noted, which Dr. LeFanu described as being of a quality such as he

had not previously heard. This bruit was most distinct in the pulmonary area, in the neighbourhood of a small scar which was present in the second intercostal space close to the edge of the sternum. There was no history or evidence of syphilis. The patient had been medically examined upon several occasions during the last year or two, and had not been informed after any of these examinations that there was anything the matter with his heart. During the war he was passed as fit for the Flying Corps, and a year before his death the Medical Adviser to the Colonial Office had passed him as fit to take up the duties of a foreman in the Public Works Department, work which is well known to entail heavy manual labour. It is, therefore, unlikely that this heart condition was of old standing, but it may be mentioned that the patient himself stated that he had always thought that his heart was affected, although he could give no history of symptoms in support of this idea. He was informed by Dr. LeFanu that his physical condition did not justify his return to West Africa, and was cautioned with regard to the risk he would run in attempting any strenuous muscular effort. Nevertheless, a few days later, as has already been stated, he fell dead on the beach when waiting to embark on the homeward-bound steamer.

At the autopsy it was found that the right arm had been amputated slightly above the middle of the shaft of the humerus, and a small scar about the size of a shilling was observed on the skin in the second left intercostal space half an inch from the sternal margin. In view of the pathological condition subsequently found, it may be stated at once that this scar was superficial, and that a careful examination failed to reveal any deep-seated injuries connected with it. Upon opening the thorax the pericardial sac was seen to be greatly distended, measuring vertically about eight inches and horizontally six and a half inches, and displaced the lungs on either side. It contained fluid blood under considerable pressure, so that on first opening it a jet of blood was projected for a distance of about a yard. About one pint of blood issued from the pericardium, and several recent clots were removed when the sac was fully opened. With the exception of the condition of the heart to be described immediately, the organs of the body appeared to be healthy.



The *heart* was not enlarged, but showed a considerable deposit of epicardial fat. Upon inverting the pericardium a discoloured, purple patch was found situated over the left auricular appendix. This patch was about as large as a broad-bean, and some tags of fibrin were adhering to it. On opening the heart the left auricular appendix was found to be dilated, its endocardium discoloured in a similar manner to the patch on the outer surface already mentioned, and its wall thin, smooth (owing to the disappearance of the pectinate arrangement of the muscles) and very friable. On examining the auricular appendix, the finger penetrated the wall and appeared in the discoloured patch on the outer surface, so that it was clear that it was through a rupture in this situation that the blood had escaped into the pericardium. With the exception of a few tiny, pearl-like vegetations at the margins of the mitral cusps on their auricular surfaces, no other abnormalities were observed in the heart or the great vessels at its base. The muscle of the walls of the ventricles appeared to be healthy, but it is to be regretted that circumstances did not permit of a detailed microscopical examination being made.

The condition of the left auricle in this case, the thinning of the wall, its friability, and the loss of its muscular rugosity, indicate that the dilatation was actually an aneurysm, and that this had finally ruptured, causing the death of the patient. According to Hall (1903), aneurysms of the chambers of the heart other than the left ventricle are 'no more than pathological curiosities, and are of the very rarest occurrence.' With regard to the left auricle, he states that Younge and Dreschfeld have each published a case of aneurysm of this cavity, and that in Younge's case, a man aged twenty-eight years, the cardiac valves were healthy, and in Dreschfeld's, a woman aged fifty-eight years, there was great stenosis of the mitral orifice. The case we have described resembled the former case more closely than the latter, for with the exception of a few small vegetations on the mitral cusps the cardiac valves were healthy. Rupture of the left auricle, indeed, appears to be a very uncommon occurrence; Odriozola (quoted by Hektoen and Riesman) recording it only in two cases in a series of one hundred and thirty-two cases of rupture of the heart. Unfortunately we are unable to make any suggestions as to the cause of the condition in our case, but so far

as the evidence went there was no reason to suppose that syphilis had anything to do with it. The detection of a remarkable bruit by Dr. LeFanu a few days before the death of the patient is of interest, since such observations are seldom made.

CASE II. A European man, aged thirty-six years, who died at Accra in February, 1922, of heart failure after an illness which had lasted about a month. His blood serum, tested on the eighth day of his illness, agglutinated *B. typhosus* in dilutions up to 1 : 125, and was negative to *B. para-typhosus* A and B; it also gave a weak positive Wassermann test and a positive Sachs-Georgi test. We are indebted to Dr. C. V. LeFanu for the following clinical account of the case.

In 1914 the patient was accepted for active service in a line regiment, served both in Gallipoli and France, and remained with the colours for five years. In 1916 he had some eye trouble, the nature of which is not known. In December, 1920, he joined the Gold Coast Service, and arrived in the Colony in the following January, so that at the time of the commencement of his last illness he had just completed a tour of twelve months and was expecting orders to return to England on leave. During his tour of service his name had appeared on the sick-list only once, namely, from the 16th of May to the 5th of June, on account of an attack of subtertian malaria. It is of interest to note that on this occasion no cardiac bruit was noted. The patient claimed to have lived a perfectly normal existence, and no history of syphilis or rheumatism was elicited.

On the 10th of January, 1922, the patient complained of fever and malaise. Two days later he was admitted to hospital. His symptoms were as follows:—Temperature 101° F. Tongue clean. Pupils strongly contracted ('pin-point') and reacting only very slightly to light. Very marked clubbing of all the finger-tips. Pulse 116, of Corrigan type; strong pulsations visible in the neck. The chest literally rocked with the cardiac action; the apex impulse was in the sixth interspace three-quarters of an inch inside the left nipple line. A rough double bruit was audible over the aortic and mitral valves, and a loud double bruit was also audible posteriorly to the left of the vertebrae, extending approximately from the seventh or eighth spinous process downwards. The radial arteries

were atheromatous. The liver was slightly enlarged downwards. The spleen extended well below the costal margin. The urine contained a trace of albumen. The action of the bowels was normal. No malaria parasites were found in the blood.

On the 18th of January a few scattered petechial spots were observed on the shoulders, chest and abdomen. Ten days later a slight, dry cough developed, followed two days later by signs of consolidation in both lungs, and subsequently by signs of pleural effusion on the right side. On the 10th of February the patient died suddenly in his sleep. The irregular fever and the oscillations of the pulse rate during the illness are shown in the chart. It may be remarked that it is somewhat strange that the gross cardiac lesions, which must have existed for years, had not previously been detected, although the patient must have been medically examined repeatedly in the Army and before coming to West Africa. With reference to the eye condition, the severity of the illness unfortunately prevented a more careful enquiry being made into the cause of the pupillary contraction which had been known to exist since 1916, and which continued unchanged throughout the illness.

At the autopsy both lungs showed broncho-pneumonic consolidation, especially of the upper lobes. The right pleural cavity contained about 30 ounces of a clear straw-coloured effusion. The liver was depressed, and was also considerably enlarged and in a state of chronic venous congestion. The spleen was enlarged and congested. In addition to the condition of the heart, presently to be described, the pericardium contained a small excess of fluid, but there were no adhesions.

The *heart* (Plate X, and text-fig.) was hypertrophied, weight about 17 ounces. The right side was slightly dilated, but otherwise presented no gross abnormalities. The wall of the left ventricle was hypertrophied, but appeared to be healthy. The aortic valves bore firm vegetations, apparently of some considerable age; the first part of the aorta was the seat of extensive atheromatous disease, and the orifices of the coronary arteries were patulous. The principal lesion was found, however, in the anterior cusp of the mitral valve. From the middle of this cusp, projecting towards the auricle, was a large aneurysmal sac which had ruptured, leaving a wide, irregular opening. The diameter of this aneurysmal sac was about 12 mm.,

and it was produced on the side directed towards the apex of the cusp into a rounded process measuring about 10 mm. in length and 9 mm. in thickness. Round the ragged margin of the ruptured portion of the aneurysm were a few small vegetations, and in the marginal portions of the cusp not involved in the aneurysmal dilatations were numerous small, white, thickened areas. Sections of the wall of the left ventricle appeared to be almost normal; there

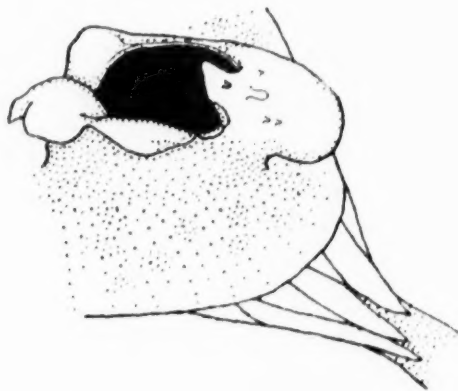


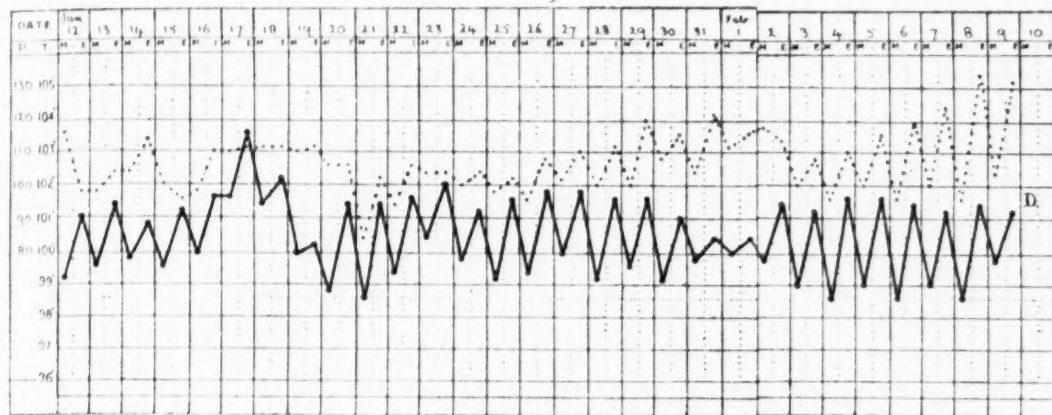
FIG. Case II. Sketch showing the ruptured aneurysmal sac in sub-lateral view.

was, however, a slight increase of the interstitial fibrous tissue. The coronary arteries showed a high degree of endarteritis. In the accompanying photograph (Plate X), the appearance of the aneurysm and the atheromatous condition of the aorta are fairly clearly seen, and in the figure, which is a rough sketch of the anterior cusp of the mitral valve as seen in a sub-lateral view, some of the characters of the lesion are more clearly indicated.

The site of the aneurysm in this case, the anterior cusp of the mitral valve, is that which, according to Drasch, is the more usual. The history of the case, the very pronounced clubbing of the fingertips, etc., suggests that the cardiac lesion was of old standing, but caused the patient himself no inconvenience or discomfort, and gave rise to signs so slight that they escaped detection at medical examinations such as that required in the case of officers proceeding to West Africa. The aneurysm of the mitral cusp, although of considerable size, was, in fact, in such a situation that it would not necessarily interfere with the efficient closing of the valve. The rupture of the aneurysm at its apex was, it may be supposed, the immediate cause of the sudden development of cardiac symptoms,



and the supervention of pneumonic infection the determinate cause of death. The irregular fever, shown in the chart, before the onset of the pulmonary complications is suggestive of endocarditis, but we did not succeed after death in cultivating any pathogenic organism



CASE II. Chart of temperature (continuous line) and pulse (dotted line).

from the margin of the aneurysm. The results of the Wassermann and Sachs-Georgi tests, and the diseased state of the aorta, point to the probability that in this case the aneurysm had developed as the result of atheromatous processes.

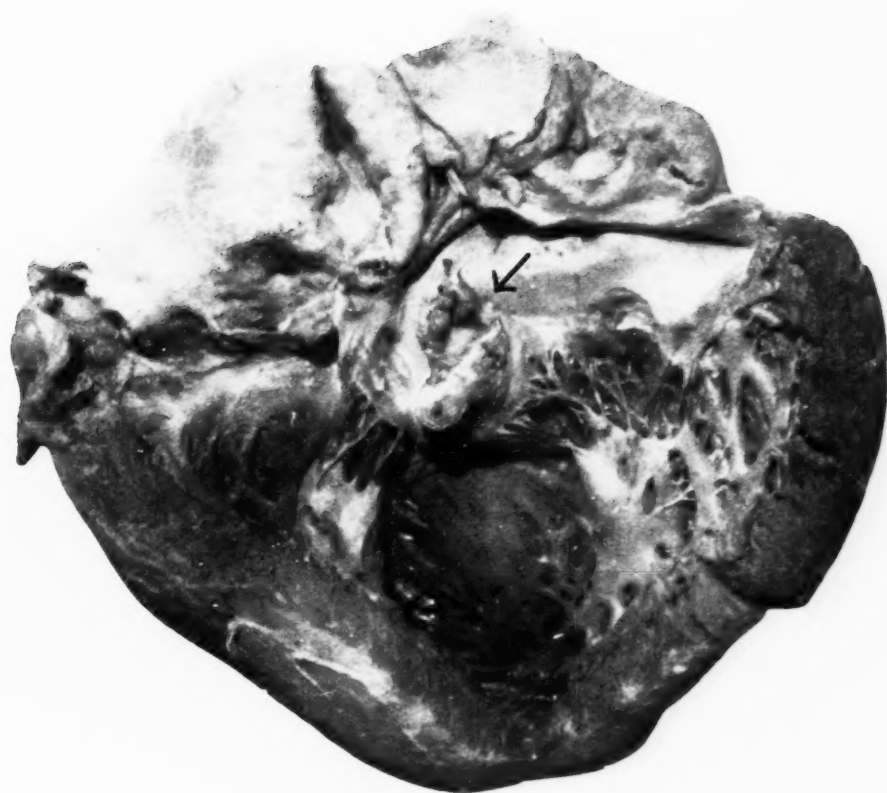
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EXPLANATION OF PLATE X

Aneurysm of the anterior cusp of the mitral valve of the heart.





# CESTODES IN THE COLLECTION OF THE INDIAN MUSEUM

BY

T. SOUTHWELL

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## A. MAMMALS

The parasites described below were obtained, with a few exceptions, from animals which died in the Zoological Gardens, Calcutta.

I do not propose dealing in this paper with the synonymy of the forms recorded.

Family *TAENIIDAE*, Ludwig, 1886

*Taenia crassicollis*, Rudolphi, 1810

1. One specimen from a cat. Punjab Civil Veterinary College, Lahore, 30.1.14.
2. Three complete specimens from the intestine of *Felis viverrina*. Zoological Gardens, Calcutta, collected by the author, 11.11.14.
3. Another specimen without a head, collected from the same host by the author, 17.2.16.
4. One specimen from the same host. Tollygunge, Calcutta, 9.4.20.

*Taenia serrata*, Goeze, 1782

1. One specimen from *Felis tigris*. Sukna, Darjeeling district, Bengal, 17.3.17.
2. Two complete and mature specimens from same host, shot at Sevoke, Darjeeling district, Bengal, 3.2.17.

*Taenia pisiformis* (Bloch, 1780), Gmelin, 1790

One complete specimen from intestine of *Felis leo*. Zoological Gardens, Calcutta, collected by the author, 3.1.16.

*Taenia* sp.

Eight specimens from the intestine of *Felis pardus*. Zoological Gardens, Calcutta, collected by the author, 19.1.15.

The specimens measured about 1 cm. long and 0.5 mm. broad; they were all immature, no trace of genitalia being visible.

The head was armed with a double row of hooks, the number varying from thirteen to seventeen in each row. The large hooks measured from  $135\mu$  to  $145\mu$ , and the smaller from  $90\mu$  to  $100\mu$ .

About fifty segments only were present; the neck measured about  $750\mu$ . As the specimens were quite immature, it was impossible to say whether they were new or not.

The following species have been recorded from:—

(1) *Felis concolor*

(a) *T. ammonitiformis*, Baird, 1862, possesses only a single row of hooks.

(b) *T. oligantha*, Diesing, 1863, has only three to four segments.

(2) *Felis pardus*

(a) *T. polycalcaria*, Linstow, 1903, possesses two rows, each row with nineteen hooks, measuring  $238\mu$  and  $158\mu$ .

(b) *T. serrata*, Goeze, 1782, has two rows each with twenty to twenty-one hooks, which measure  $250\mu$  to  $260\mu$  and  $150\mu$  to  $155\mu$ .

It is, of course, quite probable that in the worm in question the hooks would have increased in size as it matured, and that it may be either of the last two species.

*Taenia* sp.

Fragments comprising a few segments (all sterile) of what appeared to be a *Taenia* sp. were obtained from the intestine of a dog at Lahore. No date.

*Taenia* sp.

Fragments from the intestine of *Felis tigris*. Zoological Gardens, Calcutta, collected by the author, 22.2.16.

No head was present and no gravid uterus, hence the determination of these fragments was impossible, but superficially they resembled segments of *T. serrata*.



*Taenia* sp.

One specimen without head from the intestine of *Ursus torquatus* (bear). Zoological Gardens, Calcutta, collected by the author, 21.9.15.

The worm measured about 1 m.; the segments at the anterior end were square and mature. The genital pore was prominent, and, in gravid segments, was situated posterior to the middle of the segment. The worm resembles *T. pisiformis* externally, but the eggs are round and measure  $40\mu$  to  $45\mu$ ; those of *T. pisiformis* are oval and measure about  $37\mu$  by  $32\mu$ .

*Cysticercus fasciolaris*, Rudolphi, 1808

1. *Mus rattus*. Berhampore, Bengal. Collected by Lt.-Col. Clayton Lane and numbered Z.E.V.  $\frac{5416}{7}$  in the collection of the Indian Museum. No date.

2. *Mus decumanus*. Collected by Lt.-Col. Alcock and numbered Z.E.V.  $\frac{2367}{7}$  in the collection of the Indian Museum. Locality and date not given.

3. Rat. Civil Veterinary College, Lahore. Numbered Z.E.V.  $\frac{4672}{7}$  in the collection of the Indian Museum. No date or locality given.

4. Liver of rat. Collected by Dr. D. E. Muir. No date or locality given.

5. *Mus rattus*. Calcutta. Collected by Lt.-Col. Clayton Lane. Numbered Z.E.V.  $\frac{927}{7}$  in the collection of the Indian Museum. No date.

*Cysticercus cellulosae* (Gmelin, 1790), Rudolphi, 1808

One specimen from human brain, Colombo, collected by the author, June, 1911.

*Cysticercus tenuicollis*, Rudolphi, 1810

Four specimens from the four-horned antelope (*Tetracercus quadricornis*). Zoological Gardens, Calcutta, collected by the author, February, 1914.

*Cysticercus* sp.

Collected by Capt. Boulenger, 14.12.18. Host and locality unknown.

Family *HYMENOLEPIDIDAE*, Railliet and Henry, 1909

Sub-family (1) *HYMENOLEPIDINAE*, Ransom, 1909

*Hymenolepis murina* (Duj., 1845), R. Blanchard, 1891

A few specimens from the following sources:—

1. No history. Numbered Z.E.V.  $\frac{4689}{7}$  in the collection of the Indian Museum.
2. From a rat. Civil Veterinary College, Lahore, Punjab, no date. Numbered Z.E.V.  $\frac{4672}{7}$  in the collection of the Indian Museum.
3. From *Mus decumanus*, collected by Lt.-Col. Alcock, I.M.S., Calcutta. No date. Numbered Z.E.V.  $\frac{2367}{7}$  in the collection of the Indian Museum.

*Hymenolepis diminuta* (Rudolphi, 1819), R. Blanchard, 1891

1. A few specimens from the intestine of a rat, London. Numbered W.  $\frac{16}{1}$  in the collection of the Indian Museum.
2. A few specimens from the intestine of *Mus rattus*, Hong Kong, collected by Capt. F. H. Stewart, I.M.S., and numbered W.  $\frac{17}{1}$  in the collection of the Indian Museum. No date.

Sub-family (2) *DIPYLIDIINAE*, Stiles, 1896

*Dipylidium caninum* (Linn., 1758), Railliet, 1892

1. From a cat, Egypt. Numbered Z.E.V.  $\frac{2979}{7}$  in the collection of the Indian Museum. No date.
2. From the intestine of a cat, Punjab Civil Veterinary College, Lahore, 30.1.14.
3. From the intestine of a dog. Numbered Z.E.V.  $\frac{5505}{7}$  in the collection of the Indian Museum. Locality and date not given.
4. From the intestine of a dog, Lahore. No date. Numbered Z.E.V.  $\frac{4675}{7}$  in the collection of the Indian Museum.
5. From the intestine of a dog, Ceylon Medical College, Colombo. Numbered Z.E.V.  $\frac{5507}{7}$  in the collection of the Indian Museum. No date.
6. Several specimens. Locality, host, and date not given. Numbered Z.E.V.  $\frac{2979}{7}$  in the collection of the Indian Museum.

7. Two specimens from the intestine of *Felis viverrina*. Zoological Gardens, Calcutta, 23.5.19.

8. Three specimens from the intestine of *Hyaena striata*. Zoological Gardens, Calcutta, collected by the author, 17.8.15.

9. Several specimens from *Paradoxurus grayi* (Himalayan palm-civet). Zoological Gardens, Calcutta, collected by the author, 29.3.15.

*Dipylidium gervaisi*, Setti, 1895

1. One specimen from the intestine of *Felis viverrina*. Zoological Gardens, Calcutta, 30.5.19.

2. Several specimens from the intestine of *Paradoxurus hermaphroditicus* (Malayan palm-civet). Zoological Gardens, Calcutta, collected by the author, 18.5.15.

#### Family ANOPOLOCEPHALIDAE, Führmann, 1907

##### Sub-family ANOPOLOCEPHALINAE, Blanchard, 1891

*Anoplocephala vulgaris*, Southwell, 1920

One specimen from *Rhinoceros sondaicus*. No date or locality. Numbered Z.E.V.  $\frac{4680}{7}$  in the collection of the Indian Museum.

From a superficial examination of this worm in 1916, I was led to the opinion that it probably belonged to the genus *Thysanosoma*. A more careful examination of the anatomy has, however, left no doubt that it is an *Anoplocephala*, identical with the species *vulgaris*.

*Bertiella satyra* (R. Blanchard, 1891), Stiles and Hassall, 1902

One specimen without head, from the intestine of *Simia satyrus*. Zoological Gardens, Calcutta, collected by the author, 5.4.16.

*Cittotaenia mosaica*, Hall, 1908

A few specimens from *Lepus ruficaudatus*, Songara, Gonda district, United Provinces, India. Museum collector (R. Hodgart). Numbered Z.E.V.  $\frac{2771}{7}$  in the collection of the Indian Museum. As a result of a preliminary examination, this species was identified as *C. bursaria*, Linstow, 1906. More careful examination of prepared

slides left no room for doubt that they are identical with Hall's specimens.

*Moniezia trigonophora*, Stiles and Hassall, 1892

1. An immature specimen from the intestine of a black buck (*A. cervicapra*). Zoological Gardens, Calcutta, collected by the author, 30.8.13. Numbered Z.E.V.  $\frac{6044}{7}$  in the collection of the Indian Museum.

2. One specimen from the intestine of a four-horned antelope (*Tetracercus quadricornis*). Zoological Gardens, Calcutta, collected by the author, 19.8.19.

*Moniezia oblongiceps*, Stiles and Hassall, 1893

One specimen from the intestine of a domestic goat, Rangoon, Burma, collected by Dr. A. A. Marshall, 8.8.16.

*Moniezia alba* (Per., 1879), R. Blanchard, 1891

1. A few specimens from the intestine of *Bos grunniens* (Yak), Tibet, 26.6.16.

2. Other specimens of this species were obtained from sheep, Civil Veterinary College, Lahore, Punjab, 31.1.14.

*Moniezia expansa* (Rudolphi, 1810), R. Blanchard, 1891

One specimen from the intestine of a domestic goat, Rangoon, (*cercus quadricornis*). Zoological Gardens, Calcutta, collected by the author 1.2.13, and numbered Z.E.V.  $\frac{6160}{7}$  in the collection of the Indian Museum.

*Moniezia neumanni*, Moniez, 1891

One specimen from the intestine of a sheep. Civil Veterinary College, Lahore, Punjab, 31.1.14.

*Avitellina centripunctata* (Riv., 1874), Gough, 1911

Numerous specimens from cattle. Civil Veterinary College, Lahore, Punjab. No date.

*Stilesia globipunctata* (Riv., 1874), Railliet, 1893

Numerous specimens from sheep. Civil Veterinary College, Lahore, Punjab, 31.1.14.

Family *DIBOTHRIOCEPHALIDAE*, Lühe, 1902*Bothriocephalus maculatus* (Leuckart, 1848), Lühe, 1899

Very numerous specimens, all immature, measuring about 10 cms. long and 1.5 mm. broad, from the intestine of *Felis pardus* (black leopard). Zoological Gardens, Calcutta, collected by the author, 31.12.14.

*Bothriocephalus sulcatus* (Molin, 1858), Linstow, 1878

Two small specimens measuring about 10 cms. long and 3 mm. broad, from the intestine of *Felis pardus*. Zoological Gardens, Calcutta, collected by the author, 5.2.14.

*Bothriocephalus decipiens* (Diesing, 1850), Lühe, 1899

1. Very numerous specimens (mostly just mature), from the intestine of *Felis tigris*. Zoological Gardens, Calcutta, 23.2.19.

2. Another specimen without head, which appeared to belong to this species, was obtained from the intestine of *Felis pardus*. Zoological Gardens, Calcutta, collected by the author, 10.2.16.

*Bothriocephalus* sp.

One specimen from a black leopard. Collected by the author, 12.5.13.

The specimen measured 2 cms. long and its greatest breadth was 1.2 mm. As it was quite immature, it is impossible to assign it to any particular species.

*Bothriocephalus* sp.

From *Paradoxurus grayi* (Himalayan palm-civet). One specimen 10 cms. long and 6 to 7 mm. wide. No head. Zoological Gardens, collected by the author, 19.2.16.

Order *TETRAPHYLLIDEA*, Carus, 1863Genus *Ophiotaenia*, La Rue, 1911

The systematic position of this genus within the above order is a matter of some uncertainty.



*Ophiotaenia punica* (Cholodkovski, 1908), La Rue, 1911

Four specimens (one immature), from *Paradoxurus hermaproditicus* (Malayan palm-civet). Zoological Gardens, Calcutta, collected by the author, 18.5.15.

The largest specimen measured about 30 cms. long and 4 mm. broad. The cirrus was spiny; otherwise the worm agreed in detail with the description of this species given by La Rue.

Cholodkovski obtained the parasite from a dog in Tunis (1908); Hall, Ransom and La Rue were all of opinion that the normal host is a snake, and that the presence of the worm in a dog was to be accounted for by the dog having eaten a snake. On this hypothesis we have to assume that the Malayan palm-civet must likewise have eaten a snake which harboured the adult worm, but its presence in both a dog and a cat, each from different localities, is of note.

*Cestoda* sp.

About ten segments of a worm from the intestine of *Loris gracilis*. Zoological Gardens, Calcutta, collected by the author, 29.7.16. They measure about 2 mm. wide and are much broader than long. The genital pores are irregularly alternate. The ovary is central, anterior and fan-shaped, the testes being posterior and extending across the segment. The cirrus is unarmed. Eggs round and measuring  $35\mu$ , not in capsules; they have double coverings and contain a hexacanth embryo. Pyriform apparatus absent. Owing to lack of material and the absence of a head, it is impossible to say with certainty to which genus the specimens belong.

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## B. PIGEONS

*Moniezia columbae*, Führmann, 1902

= *Paronia carrinoides*, Diamare, *ex parte*

One specimen, without head, consisting of ripe, but not gravid, segments, from a pigeon (sp. ?), Berhampur, Bengal, India, 29.4.11.

*Davainca spiralis*, Baczynska, 1914

1. One specimen from a common pigeon (*Columba* sp.). Zoological Gardens, Calcutta, India, 25.4.19.

The uterus was not developed, but the prostatic glands were well defined. The specimen was mounted.

2. Two complete specimens, two large worms without heads and ten fragments were obtained from the intestine of *Crocopus phoenicopterus*. Zoological Gardens, Calcutta, India, collected by the author, 28.1.16.

Our specimens agreed with Baczynska's description, except in the matter of length. Whilst the types measured only 3 to 4 cms. long, our specimens measured 15 cms. long, the posterior 12 cms. being composed of gravid segments only.

3. About eight specimens of this species from intestine of a common pigeon, *Columba* sp. Zoological Gardens, Calcutta, 11.12.20.

In these specimens the number of testes varied between twelve and twenty, the greater number being invariably situated on one side.

*Davainca anatina*, Führmann, 1908

1. Four specimens from a pigeon (*Columba* sp.). Chilka Lake, Orissa, collected by the author. No date.

This species has hitherto only been recorded from *Anas boschas* dom.

2. An immature worm with a head and a few fragments, probably of this species, obtained from *Crocopus phoenicopterus* (green pigeon). Chilka Lake, Orissa, India (Chilka Survey), 22.11.14.

*Davainea ceylonica*, Bacz, 1914

1. Several fragments, without head, from *Crocopus phoenicopterus*. Zoological Gardens, Calcutta, India, collected by the author, 8.1.14.
2. Several worms, without heads, from *Columba leuconata*, Vig. (white-bellied pigeon). Zoological Gardens, Calcutta, India, collected by the author, 1.5.15.
3. One specimen from *Crocopus phoenicopterus*. Chilka Lake, Orissa, India (Chilka Survey), 22.11.14.

*DAVAINEA FÜHRMANNI*, n. sp.

1. Several complete specimens from *Crocopus phoenicopterus* (green pigeon). Zoological Gardens, Calcutta, Bengal, India, collected by the author, 26.1.14.
2. Numerous complete specimens from same host. Zoological Gardens, Calcutta, collected by the author, 22.7.15.
3. About nine specimens from same host. Zoological Gardens, Calcutta, India, collected by the author, 10.1.17.
4. About ten specimens of this worm were obtained from same host. Zoological Gardens, Calcutta. No date.
5. About twelve specimens and a large number of fragments from *Crocopus phayrai* (green pigeon). Zoological Gardens, Calcutta, India, collected by the author, 1.1.18.

## EXTERNAL ANATOMY

The largest specimen was about 80 mm. long and 0.7 mm. broad. The worms exhibited very considerable variations; in young segments the pore was situated at the extreme anterior margin, whilst in mature and gravid segments it was slightly in front of the middle.

The segments varied in shape; in some worms they were all broader than long, except the last few, which were square; in other specimens the segments were somewhat bell-shaped, whilst in still other worms the terminal segments were twice as long as broad.

The longest posterior segment measured 1.2 mm. long and 0.7 mm. broad. The genital pores are unilateral.

*Head.* The average size of the head was about  $250\mu$  broad and  $330\mu$  long. The large rostellum, which was about  $100\mu$  long and  $150\mu$  broad, is armed with a double row of about one hundred and ten hammer-shaped hooks (fig. 2), measuring from  $25\mu$  to  $30\mu$ , the

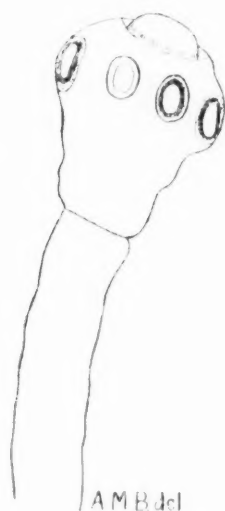


FIG. 1. *Davainea fubrmanni*, n.sp.  
Showing head and neck.  $\times 70$ .

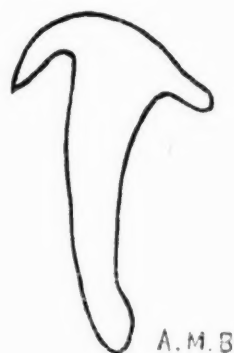


FIG. 2. *Davainea fubrmanni*, n.sp.  
Hook from the rostellum.  $\times 1,125$ .

hooks in the anterior row being slightly larger than those in the posterior row. The suckers have a diameter of about  $70\mu$  and are armed with several rows of minute hooks (fig. 1). In six of our specimens no trace of hooks was to be found on the suckers; they had apparently fallen off.

*Neck.* The neck varied in length from 0.3 mm. to 1.4 mm.

*Nervous system.* There is a single nerve situated lateral to the ventral water vessel and ventral to the cirrus pouch.

*Muscular system.* The longitudinal muscles are well-developed; the bundles are arranged in a single layer, the external being smaller in every way than the internal bundles; the arrangement is best seen in young adults. The circular fibres consist of a very narrow layer lying immediately internal to the longitudinal fibres. Oblique fibres were very scanty.



*Water vascular system.* A single ventral vessel runs along each lateral margin; that on the pore side lies ventral to the cirrus pouch and is situated further from the lateral margin than is the aporal vessel. This asymmetry is not, however, always pronounced.

#### INTERNAL ANATOMY

*Male genitalia. Testes.* The testes lie dorsal, and are about twelve in number; seven or eight lie on the aporal side of the ovary, one or two lie posterior and lateral to the yolk gland, and the rest—usually three—lie on the pore side of the ovary. They do not extend beyond the water vessels (figs. 3 and 4).

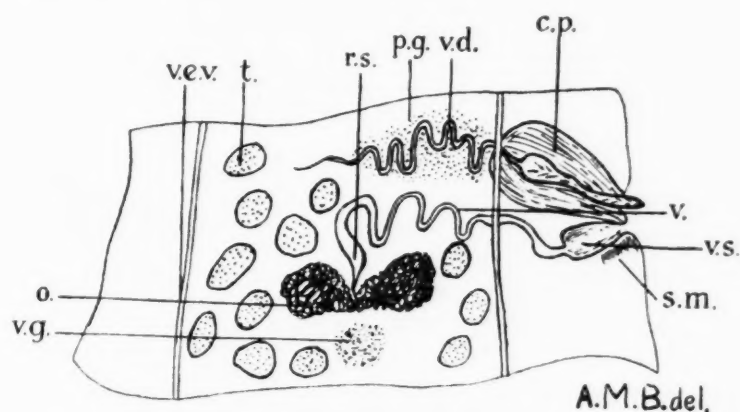


FIG. 3. *Davainea fubrmanni*, n.sp. Segment showing male and female genitalia. c.p.—cirrus pouch; o.—ovary; p.g.—prostatic gland; r.s.—receptaculum seminis; s.m.—sphincter muscle; t.—testes; v.—vagina; v.d.—vas deferens; v.s.—vaginal sinus; v.e.v.—ventral excretory vessel; v.g.—vitelline gland.  $\times 140$ .

*Vas deferens.* The vas deferens is a long, loosely coiled, slightly dilated tube, extending quite half way across the segment and surrounded throughout its length by a dense mass of glandular tissue—the prostate gland; it reaches its full development somewhat late. As no seminal vesicle was observed, it would appear that the elongated vas deferens functions as a seminal vesicle. The cirrus pouch is large, measuring in mature segments about  $170\mu$  long and  $80\mu$  broad: it lies across the antero-lateral angle of the segment and extends just internal to the lateral water vessel. The cirrus is armed with large spinules, measuring about  $17\mu$ ; these, however, cannot always be seen (figs. 3 and 4).

*Female genitalia. Ovary.* The ovary is bi-lobed, each lobe having a rounded appearance. It lies slightly behind the centre of the segment (figs. 3 and 4).

*Receptaculum and vagina.* The vagina is a long, muscular, sinuous tube; the terminal portion lying posterior to the whole length of the cirrus pouch is often, but not always, dilated. Its extreme lateral extremity lies at the base of a well pronounced sinus, situated immediately posterior to the cirrus pouch; a well developed sphincter muscle surrounds the opening of the vaginal sinus. Slightly anterior to the ovary the vagina dilates into a small but somewhat elongated receptaculum (figs. 3 and 4).

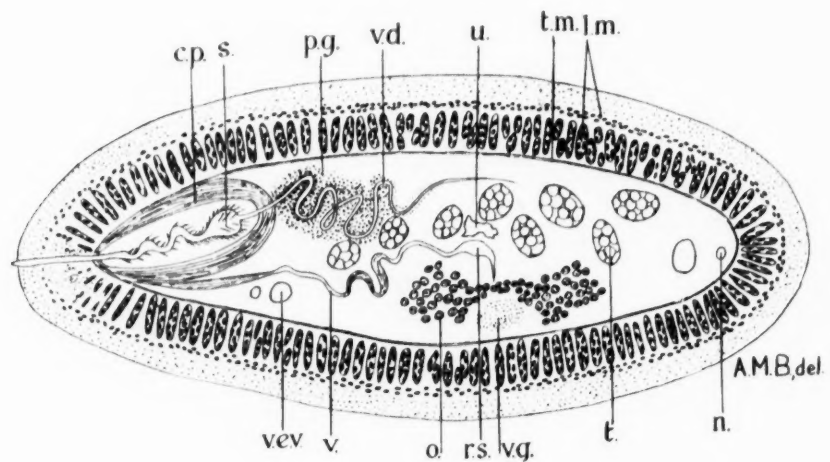


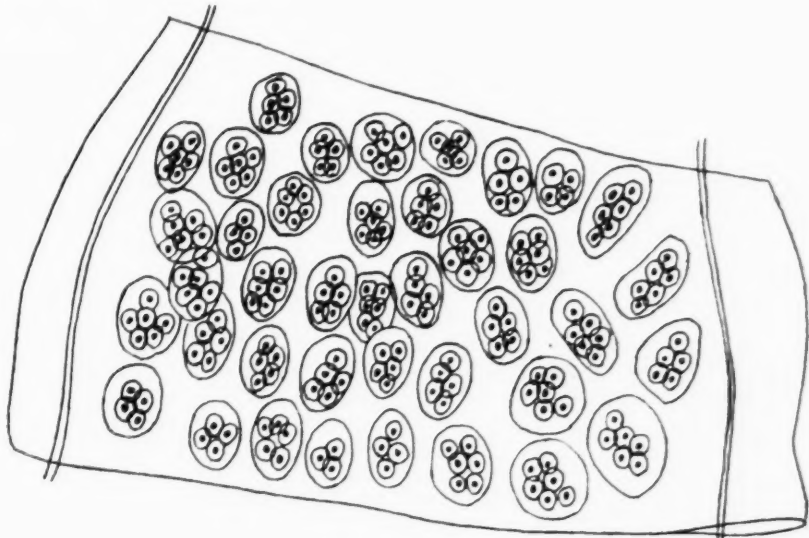
FIG. 4. *Davainea fübmanni*, n.sp. Transverse section showing male and female genitalia, etc. *c.p.*—cirrus pouch; *l.m.*—longitudinal muscle; *n.*—nerve; *o.*—ovary; *p.g.*—prostatic gland; *r.s.*—receptaculum seminis; *s.*—spines on cirrus; *t.*—testes; *t.m.*—transverse muscle; *u.*—uterus; *v.*—vagina; *vd.*—vas deferens; *vev.*—ventral excretory vessel; *vg.*—vitelline gland.  $\times$  about 160.

*Vitelline glands.* This lies posterior to the ovary, and is easily seen. In size it is almost equal to one wing of the ovary (figs. 3 and 4).

*Uterus.* The uterus is first visible as a small, irregular cavity, situated immediately anterior to the vitelline gland. It enlarges rapidly, eventually filling the entire segment between the water vessels. The eggs, when first seen, appear as a dense granular mass filling the uterus. A few segments further back about forty capsules are differentiated, each containing six or seven, and rarely nine to eleven, oncospheres. At first the mature uterus lies strictly within the water vessels, but in the last five or six segments, the water vessels disappear and the entire segment is occupied by the capsules

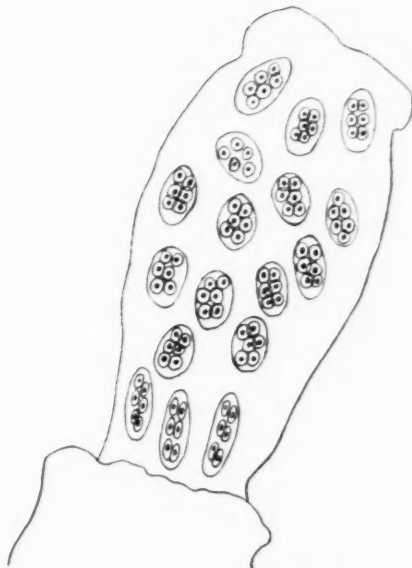
(figs. 5 and 6). Black pigment occurs abundantly in the posterior two-thirds of the worm.

*Eggs.* These measure about  $36\mu$ .



A.M.B. del.

FIG. 5. *Davainea fübmanni*, n.sp. Gravid segment showing eggs in capsules.  $\times 120$ .



A.M.B. del.

FIG. 6. *Davainea fübmanni*, n.sp. Gravid segment showing eggs in capsules.  $\times 60$ .

#### DIAGNOSIS

The species noted below have been recorded from *Columbiform* birds, and the table shows the principal points in which they differ from *D. fübmanni*.

	Length	Breadth	No. of hooks	Size of hooks	Pores	No. of testes	No. of eggs per capsule
<i>D. goura</i> ... ..	mm. 170	mm. 1.1	300	9 $\mu$	unilat.	18-20	8-10
<i>D. cryptacantha</i> ... ..	120	1.5	170	7 $\mu$	"	8-12	several
<i>D. spiralis</i> ... ..	30-40	1.3	300	15.6 $\mu$	"	4-5	4-6
<i>D. paucitesticulata</i> ... ..	100	0.6	120	9-10 $\mu$	"	6-7	7-8
<i>D. insignis</i> ... ..	300	?	?	?	"	?	?
<i>D. micracantha</i> ... ..	100	0.8	160-200	13-14 $\mu$	"	12-16	4-5
<i>D. columbae</i> ... ..	70	1.0	120	11 $\mu$	irreg.	30	1
<i>D. crassula</i> , Führ. ... ..	250-400	4.0	70	20 $\mu$	"	30-40	3-4
"    Clerc. ... ..	?	?	400	10 $\mu$	?	?	?
"    Stiles ... ..	?	?	70	10 $\mu$	?	?	?
<i>D. führmanni</i> , n.sp. ... ..	80	0.7	110	25-30 $\mu$	unilat.	About 12	6-7

I have been unable to obtain a description of *D. insignis* (Steud), but, according to Meggitt, it has armed suckers. The only species of *Davainea* possessing hooks about 28 $\mu$  long are *D. mutabilis*, *D. campanulata*, *D. undulata* and *D. vaganda*. In the first two the pores are alternate and the suckers unarmed: *D. vaganda*, Baylis, has only six to eight testes. Führmann informs me that his species *D. undulata* is different from *D. führmanni*, n. sp.

Our worm bears a general resemblance to *D. allomyodes*, Kotlán, 1921, especially in the following particulars:—

1. Size.
2. Unilateral pores.
3. The cirrus pouch and armed cirrus.
4. The vaginal sinus and sphincter.
5. Number of testes.
6. Number of eggs in each capsule.

It differs from *D. allomyodes* in the following respects:—

1. Size and number of hooks (one hundred and sixty to two hundred in *D. allomyodes*, measuring 17 $\mu$  to 18 $\mu$ ).
2. No mention is made in the description of *D. allomyodes* of the very long, loosely coiled vas deferens.
3. Number of capsules per segment, viz., sixteen in *D. allomyodes* and at least forty in *D. führmanni*, n. sp.

Some variations in the number of capsules in each segment is to be expected, but in this case the difference is considerable.

Our species is, however, much more closely related to *D. ceylonica*, Bacz., 1914, obtained from *Pavo cristatus* in Ceylon, the very long vas deferens being thrown into loops in both species. They appear to differ, however, in the following characters:—

		Length	Breadth	Size of hooks	Spines on cirrus	Ovary	Vaginal sinus
<i>D. fübmanni</i>	... ..	mm. 80	mm. 0.6-0.7	25-30 $\mu$	Present	Bi-lobed	Present
<i>D. ceylonica</i>	... ..	30-40	1.3	10 $\mu$	Not described	Fan-shaped	Not described

It will be clear that the principal difference lies in the size of the hooks, which, being hard, do not alter in size as the soft structures are liable to do.

The notable characters of *D. fübmanni*, n. sp., are as follows:—

1. Large hooks on the rostellum 25 $\mu$  to 30 $\mu$ .
2. Suckers armed.
3. Long neck.
4. Pores unilateral.
5. Few testes (about twelve).
6. The large cirrus pouch.
7. Large spines on cirrus (measuring 17 $\mu$ ).
8. The very long, loosely coiled, vas deferens.
9. The large prostate gland.
10. The vaginal sinus with sphincter muscle.
11. Six or seven eggs per capsule.

I have great pleasure in naming this species in honour of Professor O. Fübmann, of the Zoological Department, University of Neuchâtel, who has contributed so much to the science of Helminthology.

#### *Davainea* sp.

A fragment, without head, from a pigeon (*Columba* sp). Berhampur, Bengal, India, 1912.



*Davainea* sp. (? *paradisea*, Führ., 1908)

A head and a few anterior segments, from a pigeon (sp. ?). Zoological Gardens, Calcutta, collected by the author, 1.2.14.

The hooks measured about  $23\mu$ , and were in a double row. The only species with hooks  $23\mu$  long are *D. paradisea*, Führ., and *D. conopophilea*, Johnstone. The specimen was mounted.

*Davainea* sp.

One specimen, from the common pigeon (*Columba* sp.). Zoological Gardens, Calcutta, collected by the author, 11.12.13.

The worm measured 15 cms. long and 3 mm. broad; head absent. The egg capsules extend beyond the water vessels, and each capsule contains three or four oncospheres.

*Hymenolepis gracilis*, Cohn, 1901

Two specimens, complete, from *Crocopus phoenicopterus*. Chilka Lake, Orissa, India (Chilka Survey), 22.11.14. Not previously recorded from this host.

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## C. DUCKS

*Hymenolepis* sp. (*sinuosa* ?, Zed., 1800), Cohn, 1901

= *T. bairdii* (Krefft, 1871)

= *H. collaris* (Batsch, 1786)

Seven specimens from intestine of *Anas poecilorhyncha*. Zoological Gardens, Calcutta, collected by the author, 6.2.15.

The worms measured 6 to 8 cms. long and the greatest breadth was 2 mm. None of the specimens possessed a head. The posterior segments were as long as broad, and some of the anterior segments were bell-shaped and much longer than broad.

The three testes were lobed. Two were situated on the aporal side, one being directly anterior to the other. The third testis was on the pore side. The ovary was situated between the testis on the pore side and the anterior aporal testis. The accessory sac was well defined.

In the absence of a head, it is impossible to say with certainty to which species our specimens belong, but they bear a very close resemblance to *H. sinuosa* (Zed.).

*Drepanidotaenia gracilis* (Zed. 1803), Railliet, 1893

Six specimens from a tufted duck (*Fuligula cristata*). Loktak Lake, Manipur, Assam, Station 15, February 22nd, 1920. Manipur Survey, Zoological Survey of India. Recorded for the first time from this host.

*Drepanidotaenia fasciata* (Rud., 1810), Railliet, 1893

Duck. Intestine. No date or other details. Madras, collected by the author.

*Fimbriaria malleus* (Goeze, 1782), Froel, 1802

One specimen from a tufted duck (*Fuligula cristata*). Loktak Lake, Manipur, Assam, Station 15, February 22nd, 1920. Manipur Survey, Zoological Survey of India. Recorded for the first time from this host.

*Cotugnia* (?) *bifaria* (Sieb., 1848), Stiles, 1896

One specimen, 80 mm. long and without head; from a duck (species ?). Zoological Gardens, Calcutta, collected by the author, 4.3.14.

*Diploposthe laevis* (Bloch, 1782), Jacobi, 1896

1. Three specimens without heads. Collected by the author from *Nyroca fuligula* (the tufted duck); Zoological Gardens, Calcutta, 28.1.16.

The worms measured about 80 mm. long and 5 mm. broad.

2. One complete specimen from *Nyroca baeri* (eastern white-eyed duck). Zoological Gardens, Calcutta, 11.4.11.

The specimen measured about 85 mm. long and 6 mm. broad. Recorded for the first time from this host.

#### D. CROWS

Genus *Davainea*, Blanchard and Railliet, 1891

*Davainea corvina*, Führmann, 1905

= *D. polycalcaria*, Linstow, 1906

##### (a) From *Corvus macrorhynchus*

1. Two specimens. Calcutta, India, collected by the author, September, 1912.

2. Two specimens, without heads. Calcutta, India, collected by the author, no date, and numbered Z.E.V.  $\frac{6873}{7}$  in the collection of the Indian Museum.

3. Two specimens. Calcutta, India, July 18th, 1911. Numbered Z.E.V.  $\frac{5359}{7}$  in the collection of the Indian Museum.

4. Seven specimens. Calcutta, India, collected by the author, 29.9.12.

5. Numerous specimens. Sabour, Bihar, India, collected by the author, 21.10.13.

##### (b) From *Corvus macrorhynchus* and *Corvus splendens*

6. Numerous specimens. Calcutta, India, collected by the author and numbered Z.E.V.  $\frac{6146}{7}$  in the collection of the Indian Museum.

##### (c) From *Corvus* sp.

7. Five very large specimens. Khulna, Bengal, collected by the author, 1912.

8. Numerous large specimens. Chilka Lake, Orissa, India, collected by the author, 4.8.13., and numbered Z.E.V.  $\frac{6146}{7}$  in the collection of the Indian Museum.

Genus *Hymenolepis*, Weinland, 1858

*Hymenolepis dahurica* (Linstow, 1903), Führmann, 1906

Three specimens. Calcutta, India, collected by the author and numbered Z.E.V.  $\frac{6164}{7}$  in the collection of the Indian Museum. No date.

*Cotugnia margareta*, Beddard, 1916

One specimen. Calcutta, India, collected by the author, 1913. Both the preceding species were from *Corvus macrorhynchus*.

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#### E. BIRDS (MISCELLANEOUS)

*Davainea urogalli* (Modeer), 1790

= *Taenia urogalli*, Modeer, 1790

= *Taenia calva*, Baird, 1853

= *Davainea calvi*, Shipley, 1906

Many specimens (only one with a head) from *Caccabis chucar* (Partridge). Zoological Gardens, Calcutta, India, collected by the author, 18.2.18.

*Cittotaenia avicola*, Führmann, 1897

One specimen, probably of this species, from the intestine of a moonal pheasant (*Lophophorus refulgens*). Zoological Gardens, Calcutta, India, collected by the author, 6.6.17.

The worm was complete, but in a bad state of preservation; the latter circumstance being due to the fact that the host had been dead over a day when the post-mortem was made. As a result, it was impossible to make out the anatomy with precision. The worm measured 16 cms. long, and many of the posterior segments were longer than broad; the largest measured 3.6 mm. long and 2.8 mm. broad. The head measured from 0.75 mm. to 1 mm. broad.

*Anomotaenia acollum*, Führmann, 1907

Two specimens from intestine of *Cuculus varius*. Zoological Gardens, Calcutta, India, collected by the author, 20.1.14.

*Bertia delafondi* (Railliet, 1882), Führmann, 1901

= *T. delafondi*, Railliet, 1882

= *T. sphenoccephala* (Megnin, Linstow), *ex parte*

A fragment (mounted) from intestine of *Platycercus pennanti*. Zoological Gardens, Calcutta, India, collected by the author, 25.6.15.

*Hymenolepis fusus* (Krabbe, 1869), Führmann, 1906

Numerous complete specimens from the intestine of a gull (*Larus brunneicephalus*). Zoological Gardens, Calcutta, India, collected by the author, 30.11.15.

The hooks measured  $16\mu$  and were of the shape typical in this species. The neck was long; of the three testes, two were situated on the pore side, one anterior and slightly lateral to the other.

*Dilepis macrosphincter*, Führmann, 1909

One complete specimen from the intestine of *Ardea purpurea*. Zoological Gardens, Calcutta, India, collected by the author, 20.9.15.

The specimen measured 10 cms. long and the greatest breadth was 1.7 mm. The genital pores are unilateral; the head bore sixteen to eighteen hooks measuring  $54\mu$ .

*Hymenolepis liguloides* (Gerv., 1847), Cohn, 1901

= *Amabilia lamelligera*, Linst., 1879

= *T. caroli*, Paroni, 1887



Two specimens from the intestine of *Phoenicopterus roseus* (flamingo). Zoological Gardens, Calcutta, India, 19.5.19.

*Drepanidotaenia megalorchis* (Lühe, 1898)

Several specimens from intestine of *Phoenicopterus roseus* (flamingo). Zoological Gardens, Calcutta, India, collected by the author, 16.1.13.

*Hymenolepis* ? *breviannulata*, Führmann, 1906

Fragments and one head, probably of this species, from intestine of little cormorant (*Phalacrocorax carbo*). Chilka Lake, Orissa, India. Chilka Survey. No date. Numbered Z.E.V.  $\frac{6815}{7}$  in the collection of the Indian Museum.

*Davainea cohni*, Bacz., 1914.

1. Several specimens from *Pterocles exustus*, Temm., 1825. Zoological Gardens, Calcutta, India, collected by the author, 29.1.15.

2. Six specimens, without heads, the largest measuring 16 cms. long and 1.2 mm. broad, from the intestine of *Pterocles arenarius*. Zoological Gardens, Calcutta, India, collected by the author, 24.11.16.

*Choanotaenia* (? *ungulifera*)

Several specimens, without heads, from *Totanus hypoleucus* (common sand-piper). Barkuda Island, Chilka Lake. No date.

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## F. REPTILES

Family *BOTHRIOCEPHALIDAE*, Cobb., 1864Sub-family *DIBOTHRIOCEPHALINAE*, Lühe, 1899Genus *Bothridium*, Blainville, 1824*Bothridium pithonis*, Blainville, 1828

1. Four specimens from *Python molurus*, collected by Lt.-Col. Clayton Lane, I.M.S., Darjeeling, Bengal India, 15.3.14.
2. One specimen from *Python reticulata*. Zoological Gardens, Calcutta, India, collected by the author, 26.1.18.
3. Several large specimens from intestine of *Python molurus*. Zoological Gardens, Calcutta, India, 3.7.19.
4. Several large specimens from *Python molurus*, collected by Lt.-Col. Clayton Lane, I.M.S., Berhampur, Bengal, India, May, 1913.
5. Seven very large specimens from *Python reticulata*, collected by Lt.-Col. Clayton Lane, I.M.S., Darjeeling, Bengal, India, 1917. ?
6. Two very large specimens from *Python* sp. Darjeeling, Bengal, India, 1916.

Genus *Duthiersia*, Perrier, 1873*Duthiersia fimbriata* (Diesing, 1850), Mont. and Crety, 1891

1. Several specimens from *Varanus salvator*. Outskirts of Calcutta, India. Purchased. No date.
2. Several specimens. Numbered Z.E.V.  $\frac{5364-65}{7}$  and Z.E.V.  $\frac{5451}{7}$ , from *Varanus salvator*. Zoological Gardens, Calcutta, India, collected by the author, 1914.
3. Four specimens from *Varanus salvator*. Zoological Gardens, Calcutta, India, collected by Dr. Baini Prashad, December, 1920.
4. Two specimens from *Varanus flavescens*. Zoological Gardens, Calcutta, India, collected by the author, 21.6.16.
5. A few badly preserved fragments from *Varanus flavescens*. Zoological Gardens, Calcutta, India, collected by the author, 18.7.15.
6. One specimen and a few fragments from *Varanus* sp.

Berhampur, Bengal, India, collected by Lt.-Col. Clayton Lane, I.M.S. Numbered Z.E.V.  $\frac{5508}{7}$  in the collection of the Indian Museum.

7. Five specimens from *Varanus nebulosus*. Zoological Gardens, Calcutta, India, 21.3.19

8. Several specimens from lungs, mesenteries and stomach of *Varanus salvator*. Zoological Gardens, Calcutta, India, collected by the author, 7.2.13.

9. About twelve specimens from *Varanus salvator*. Zoological Gardens, Calcutta, India, collected by the author, 19.4.13.

10. Two large and complete specimens from *Varanus nebulosus*, collected by E. Vredenburg, Esq., Geological Survey of India, 3.7.16.

11. Two small specimens from intestine of *Varanus salvator*. Zoological Gardens, Calcutta, India, collected by the author, 26.3.15.

Family *PROTEOCEPHALALIDAE*, La Rue, 1914

Genus *Acanthotaenia*, Linstow, 1903

*Acanthotaenia biroi* (Ratz, 1900)

= *Ichthyotaenia biroi*, Ratz, 1900

Numerous specimens from *V. bengalensis*, killed on the shores of the Chilka Lake, Orissa, India, collected by the author, 6.8.13. and numbered Z.E.V.  $\frac{6045}{7}$  in the collection of the Indian Museum.

The variations observed in our specimens of this species leave little doubt that it is identical with *A. tidswelli*, Johnston, separated by Johnston on account of the position of the genital pore and the shape of the cirrus pouch. A casual observation led, in the first instance, to the identification of this specimen as *I. nilotica*, Beddard, but it differs from *I. nilotica* in having only about forty-five testes, etc., whilst it agrees in all details with *I. biroi*.

Genus *Ophiotaenia*, La Rue, 1911

*Ophiotaenia* sp. (*calmetti*, Barrois ?)

Two fragments, measuring about 15 mm. long and 4 mm. broad; immature. No head present. From intestine of *Bungarus coeruleus*. Zoological Gardens, Calcutta, India, collected by the author, 7.2.18.

Genus *Ophidotaenia*, Beddard, 1913*Ophidotaenia naiae*, Beddard, 1913

One young but complete specimen from *Naia tripudians*, Zoological Gardens, Calcutta, India, 15.1.21.

The genitalia were fully developed, but the uterus, which consisted of a central stem running antero-posteriorly, was very young and not gravid. The uterine pores, described by Beddard, were therefore not developed, but in every other respect the worm agreed with Beddard's description.

Genus *Linstowia*, Zsch., 1899*Linstowia* sp.

Two specimens without heads; from *Hemidactylus flaviviridis*, killed in the grounds of the Indian Museum, Calcutta, India, collected by Dr. Baini Prashad. No date.

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## G. AMPHIBIANS

*Nematotaenia dispar*, Lühe, 1899

1. A few fragments from a toad (*Bufo* sp.), collected by Captain Stewart, I.M.S., 5.2.14.

2. One complete specimen and several fragments from intestine of *Bufo melanostictus*, collected by Captain R. B. Seymour Sewell, I.M.S.

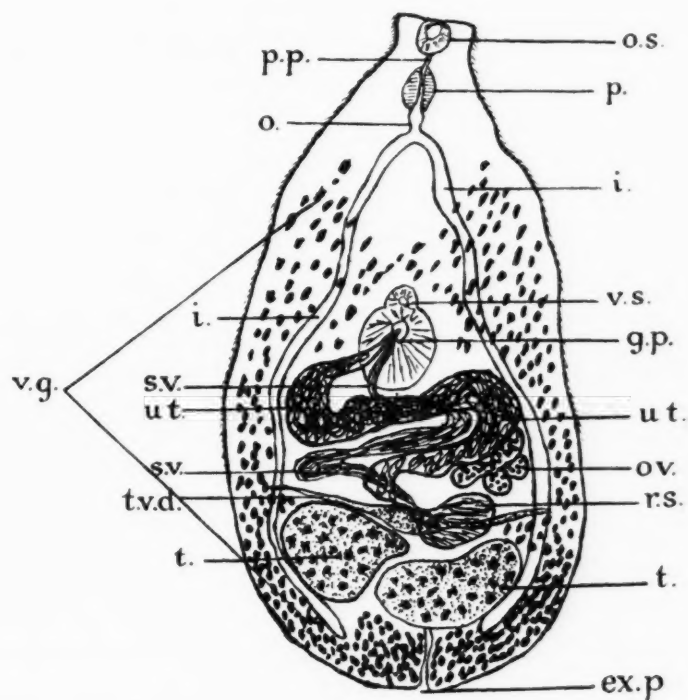
# CRYPTOCOTYLE LINGUA (CREPLIN, 1825), FISCHOEDER, 1903, IN A DOG IN ENGLAND

BY

P. A. MAPLESTONE

*(Received for publication 10 May, 1922)*

Three specimens of this small fluke were recovered from a dog killed in the Dogs' Home, Liverpool.



*Cryptocotyle lingua*, ventral view. *ex.p.*—excretory pore; *g.p.*—genital pore; *i.*—intestine; *o.*—oesophagus; *o.s.*—oral sucker; *ov.*—ovary; *p.*—pharynx; *p.p.*—prepharynx; *r.s.*—receptaculum seminis; *s.v.*—vas deferens and seminal vesicle; *t.*—testis; *t.v.d.*—transverse vitelline duct; *ut.*—uterus; *v.g.*—vitelline glands; *v.s.*—ventral sucker.  $\times 54$ .



The following table sets out the principal dimensions and anatomical characters of the three specimens:—

TABLE

	Spec. 1	Spec. 2	Spec. 3
Length and Breadth ...	1.4 × 0.7 mm.	1.3 × 0.67 mm.	1.5 × 0.75 mm.
Oral sucker ... ..	108μ	80μ	96μ
Pharynx ... ..	76μ	76 × 76μ	72 × 72μ
Oesophagus ... ..	80μ	60μ ?	? —
Bifurcation of intestine ...	260μ from anterior end	220μ from anterior end	? —
Genital sucker ... ..	—	160μ	148μ
Testes ... ..	Right testis anterior	Right testis anterior	Right testis anterior
Ovary ... ..	3 lobes in line, 260 × 68μ, on left	4 or 5 lobes grouped together about 120μ across, on left	3 or 4 lobes grouped together about 128μ across, on left
Vitellaria ... ..	Meet anterior to ventral sucker. Left side runs as far as gut fork. Right not so far	Nearly meet anterior to sucker and a few follicles about reach gut fork	Do not meet anterior to sucker and do not reach gut fork on either side
Eggs ... ..	50 × 30μ	48 × 24μ	48 × 28μ

With the exception of the relative positions of the testes and ovary, the worms closely agree with the full description given by Linton (1915). In Linton's drawing the left testis is figured as lying diagonally in front of the right testis, and the ovary is on the right side. In the present material the reverse is the case, viz., the right testis is slightly in front of the left and the ovary is on the left.

Ransom (1920), in defining the genus *Cryptocotyle*, states: 'Testes near posterior end of body, irregularly oval or globular and usually slightly lobed, or right testis obliquely behind the left. Ovary irregularly oval, or usually lobed, commonly like a clover leaf, situated on the right side of the median line in front of the seminal receptacle.' If this definition is adhered to, it will necessitate placing the present material in a new genus, which does not seem

advisable when it is so close to previously described specimens. The above points seem to be too detailed for generic distinction, especially when it is borne in mind that in other flukes of undoubtedly the same species the positions of testes and ovary frequently vary, and hence in these cases are not even of specific value. For instance, in a collection of *Fasciola hepatica* from one host, although the ovary is usually on the right, occasionally a specimen is found with it on the left; a similar instance has recently been observed by the writer in *Gastrodiscus aegyptiacus* and *Gastrodiscus secundus*. In both these species the testes are diagonally placed and either the left or right testis may be anterior, and the ovary also lies either on the right or left side, being always on the opposite side to the posterior testis (in this genus the ovary is posterior to the testes). The present variation is of exactly the same character, the ovary being on the opposite side to the anterior testis (ovary anterior to testes in the genus *Cryptocotyle*). A further reason against making the side on which the ovary lies of generic value is that Nicoll (1907), in defining the same genus, states: 'Ovary irregularly lobed, on right or left side of middle line.'

It is also apparent from the study of the present specimens that the distribution of the vitellaria is subject to considerable variation, and that Ransom's definition is too restricted in this respect. Ransom's definition, therefore, should be slightly emended so as to allow the inclusion of the present specimens in the species *C. lingua*, which appears preferable to making a new genus on admittedly variable characters.

*Occurrence.* This worm has been recorded from many fish-eating birds in Europe and North America, and once in the dog by Wigdor (1918), at Detroit, Michigan, U.S.A., as the type of a new genus *Hallum*. Ransom (1920) states that he has examined some of Wigdor's material, and he places the worm in the species *Cryptocotyle lingua*. The discovery of this worm in England makes it appear probable that it is more common in dogs than the record of its occurrence in this host would lead one to suppose, and that it has often been missed on account of its minute size.

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## ON THE GENITAL ARMATURE OF THE FEMALE MOSQUITO

BY

J. W. S. MACFIE

AND

A. INGRAM

*(Received for publication 15 May, 1922)*

So far as we have been able to ascertain, very little attention has hitherto been paid to the differential characters of the genital armature of female mosquitoes. In this paper, therefore, we record briefly the results of a preliminary examination we have made of fifty West African species referable to sixteen different genera.

It may be said generally that we have found in most cases well marked differences between distinct genera, but only slight or almost inappreciable ones between species of the same genus. The degree of resemblance is, however, very variable: in some genera, as for example in the genus *Stegomyia*, it is very close; in others, much less so. In some genera, indeed, as in the genus *Mimomyia* and the somewhat heterogeneous genus *Ochlerotatus*, there are such notable differences between certain species that they appear to be almost generic.

In making our examinations we have again found pure carbolic acid a valuable reagent. When immersed in this fluid, either with or without previous treatment with caustic potash, the abdomen of a mosquito swells out and becomes transparent, and if mounted under a cover-slip in a hollow on a glass slide, can be rolled over and over, so that every aspect of it can be carefully examined. In fresh or fairly recent preserved specimens, the spermathecae also are expanded by this treatment, so that their precise shape can be determined and accurate measurements made of their various diameters; in old specimens, however, this does not always happen as the spermathecae are collapsed, and may be so much hardened that no procedure which we have hitherto tried will restore their elasticity sufficiently to permit of their subsequent expansion when immersed in carbolic acid.

The figures illustrating this paper are mere outlines, drawn with the aid of a camera lucida, omitting both hairs and scales. It should also be explained that when giving measurements in the text, such as those of the cerci or spermathecae, the length is given first and then the breadth, unless otherwise noted. The following abbreviations are used in the figures.

- t viii, t ix.=Tergite of segment eight, nine.  
 s viii, s ix.=Sternite of segment eight, nine.  
 c. =Cerci.  
 v.p. =Ventral process of the tenth segment.  
 sp. =Spermatheca.

#### GENERAL MORPHOLOGY OF THE FEMALE ARMATURE

*External armature.* The eighth abdominal segment is usually of normal form, and has a well developed tergite and sternite; although most commonly distinct, it is sometimes partially, or even almost completely withdrawn within the seventh segment. The ninth segment, which is small and somewhat modified, is more or less withdrawn within the eighth segment, so that there is on the ventral aspect a small membranous recess in the middle of which opens the genital canal. In this recess, in the middle line immediately above the posterior margin of the eighth sternite, there is also in some species, for example in the genus *Culex*, a clearly defined, tuft-like group of stout setae. The ninth tergite is small, and is usually reduced to a narrow transverse strip of chitin bearing a few hairs, but in some genera is rather more highly developed and is shield-shaped. The genital orifice is supported by ventral sclerites which vary considerably in size and form in different species, and which are usually poorly developed. In the neighbourhood of the genital orifice, but internally and projecting forwards and downwards, is also a supporting framework which in a dorsal or ventral view appears as a more or less U-shaped structure surrounding the vulva on its lateral and posterior sides. This structure is well chitinised in some species, especially in the genus *Culex*. The tenth segment is greatly reduced, and is without either tergite or sternite. It bears dorsally the two cerci, which play a part in the manipulation of the eggs, and ventrally a short median process the function of which is apparently unknown. The cerci are more



or less leaf-like structures, and show a great diversity of size and shape. Most commonly they are short and truncated at their ends, somewhat hollowed on their inner surfaces, and set obliquely, so that their upper margins converge and a full-view of them can be obtained only when the abdomen is in a sub-lateral position. The anus opens on a membranous projection between the cerci.

*Internal armature.* The two ovaries lie one on each side of the abdomen. In recently hatched mosquitoes they are small, but in gravid individuals they occupy the greater part of the lateral and dorsal portions of the abdomen. From each ovary arises a short, wide, muscular oviduct which runs a straight course posteriorly and inwards and meets its fellow of the other side in the middle line to form the common oviduct, a relatively short, wide, muscular tube which lies ventral to the rectum and opens at the genital orifice. Into the common oviduct there opens, a short distance above the genital orifice, the duct of the gluten or mucous gland. This gland is single, and occupies a median ventral position. The spermathecae are situated on the ventral aspect of the eighth segment, and are highly chitinised oval or sub-spherical bodies enclosed in a thick cellular envelope. The chitinised wall is usually more or less pitted with small round or oval areas of thinner chitin which are most commonly grouped round the point of origin of the duct, but may be distributed over the whole surface of the spermathecae. When the spermathecae are viewed by transmitted light these areas appear as light-coloured marks, and are, therefore, referred to as 'pale spots' in the specific descriptions which follow. The ducts of the spermathecae are long and coiled, and open into the common oviduct just before the genital orifice. They are usually chitinised for a short distance only at their commencement, and for the greater part of their length appear to be muscular with an inner lining somewhat resembling a tracheal tube. In those species which have three spermathecae, the middle one is usually the larger and has an independent duct. The ducts of the lateral spermathecae unite about their middles to form a common duct which opens into the common oviduct close to, but apparently not actually in common with the duct of the middle spermatheca. The extremity of the duct of the middle spermathecae may be chitinised for a short distance before entering the common oviduct.

Sub-family *CULICINAE*

## Tribe ANOPHELINI

Genus *Anopheles*

Eight species were examined, all of which possess genitalia of a somewhat similar form. There is in each case a single, large, highly chitinised spermatheca which is sub-spherical in shape, the length being but slightly greater than the breadth. The duct of the spermatheca, which is chitinised for a short distance, arises obliquely, thus forming an acute angle with one side of the body of the spermatheca. On this side, especially near the distal pole and around the base, the chitinous wall of the spermatheca is pitted with

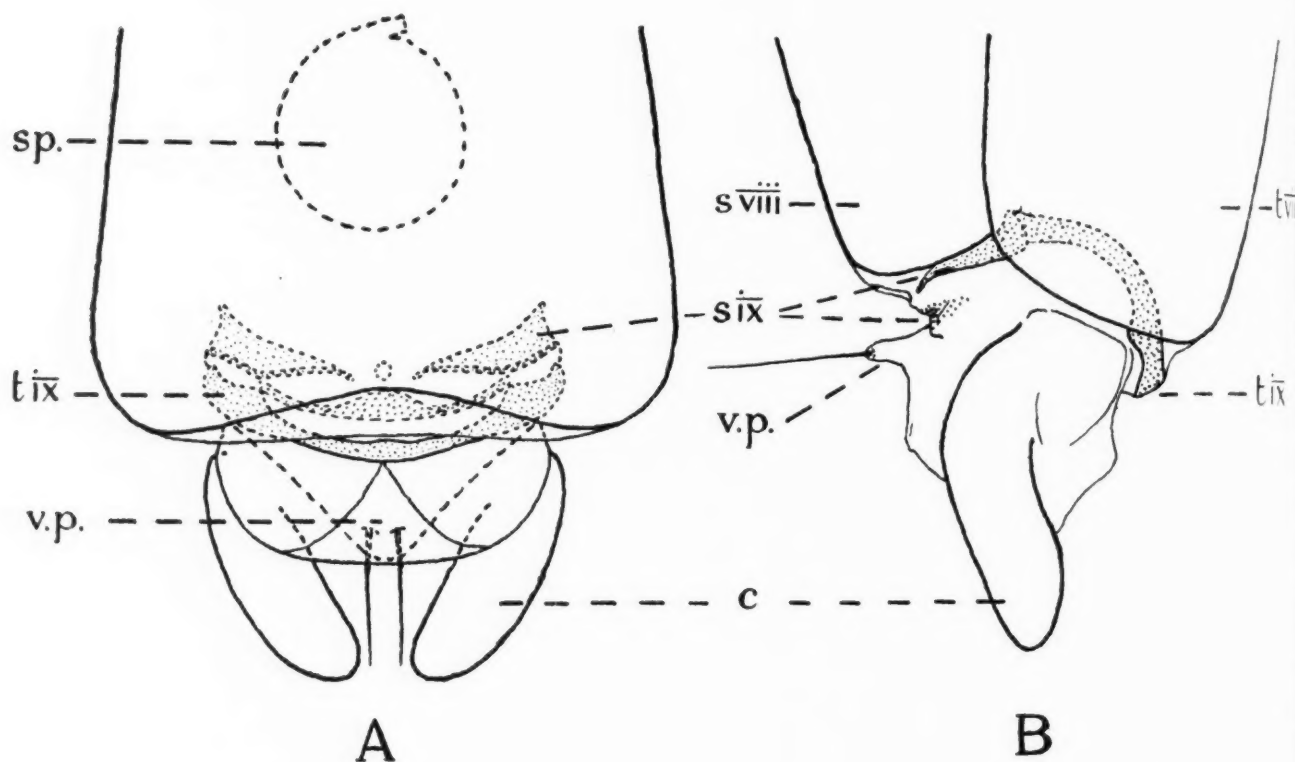


FIG. 1. *Anopheles costalis*, Theo., posterior extremity of abdomen of female. A—dorsal view; B—lateral view.  $\times$  c. 185.

numerous round or oval areas which by transmitted light appear as pale spots. The form of the spermatheca and the distribution of these pale spots furnish differential points in some species, as also do the characters of the cerci.

*A. costalis*, Theo. (fig. 1). Twenty specimens. Posterior extremity of the abdomen blunt, cerci prominent. The eighth segment not retracted within the seventh; sternite not notched in the

middle line posteriorly. The ninth segment retracted within the eighth, its tergite reduced to a narrow strip of chitin, and its sternite represented by two transverse bars of chitin which are roughly triangular in shape with their apices directed towards the middle line and a more posterior bar which is broadest in the middle line. Cerci (fig. 2 *d*) elongate-ovoid with blunt, rounded ends; length

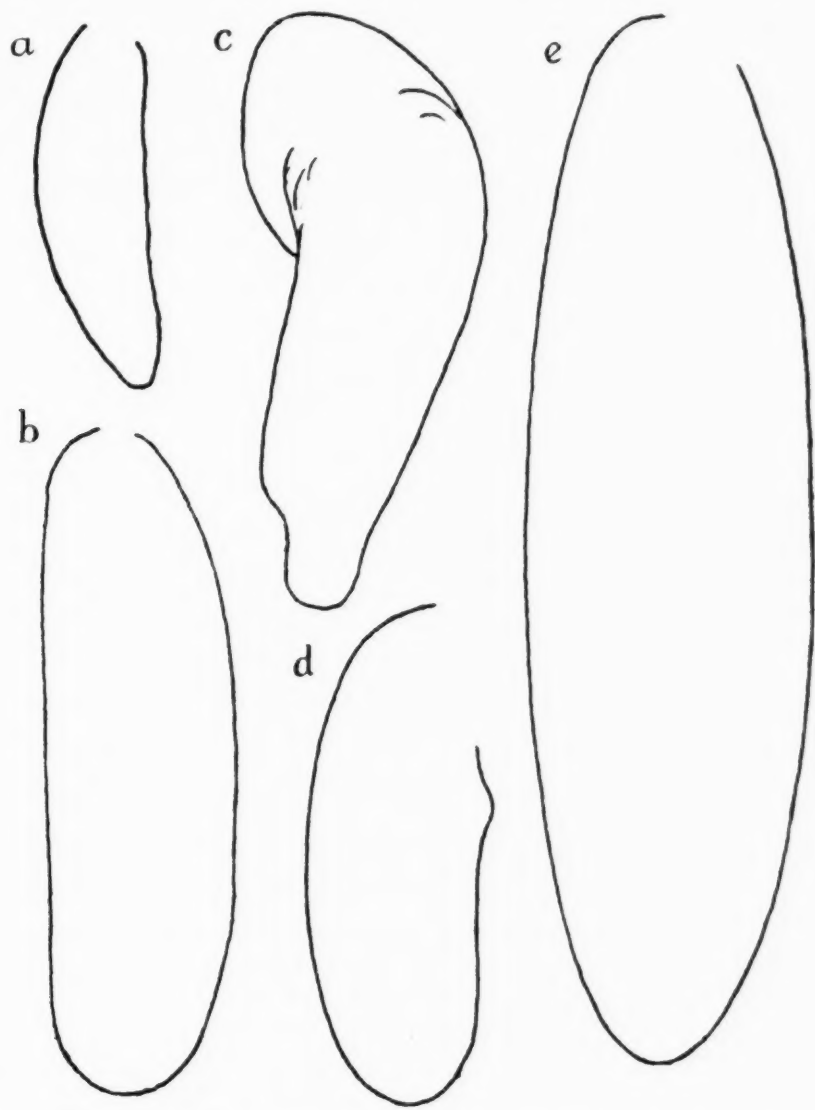


FIG. 2. Cerci, dorso-ventral views of *a*—*Anopheles nili*, Theo.; *b*—*A. squamosus*, Theo.; *c*—*A. mauritanus*, Grp.; *d*—*A. costalis*, Theo., and *e*—*A. pharoensis*, Theo.  $\times$  c. 375.

varying from  $122\mu$  to  $167\mu$ , average  $140\mu$ ; breadth from  $30\mu$  to  $62\mu$ , average  $51\mu$ . Ventral process on the tenth segment small, triangular, not emarginate, bearing two stout bristles near its apex. Spermatheca (fig. 3 *a*) single, large, sub-spherical, the length, which varied from  $95\mu$  to  $133\mu$ , average  $118\mu$ , being slightly greater than the breadth, which varied from  $95\mu$  to  $130\mu$ , average  $112\mu$ . It is

well chitinised but has numerous small round or oval areas of thinner chitin, which by transmitted light appear as pale spots, at the base and along one side, namely, the side towards which the duct projects. The duct arises obliquely, and the chitinised portion of it, which is very short and shows a few pale spots, forms an acute angle with the spermatheca; the chitinised wall of the duct lying next to the body of the spermatheca measured from  $8\mu$  to  $19\mu$ , average  $13\mu$ .

*A. marshalli*, Theo. One specimen. Generally similar to *A. costalis*. Cerci rather shorter and relatively broader, elongate-ovoid, with blunt, rounded ends; length about  $100\mu$ , breadth about  $40\mu$ . Spermatheca (fig. 3 *b*) slightly smaller, diameter about  $95\mu$ ;

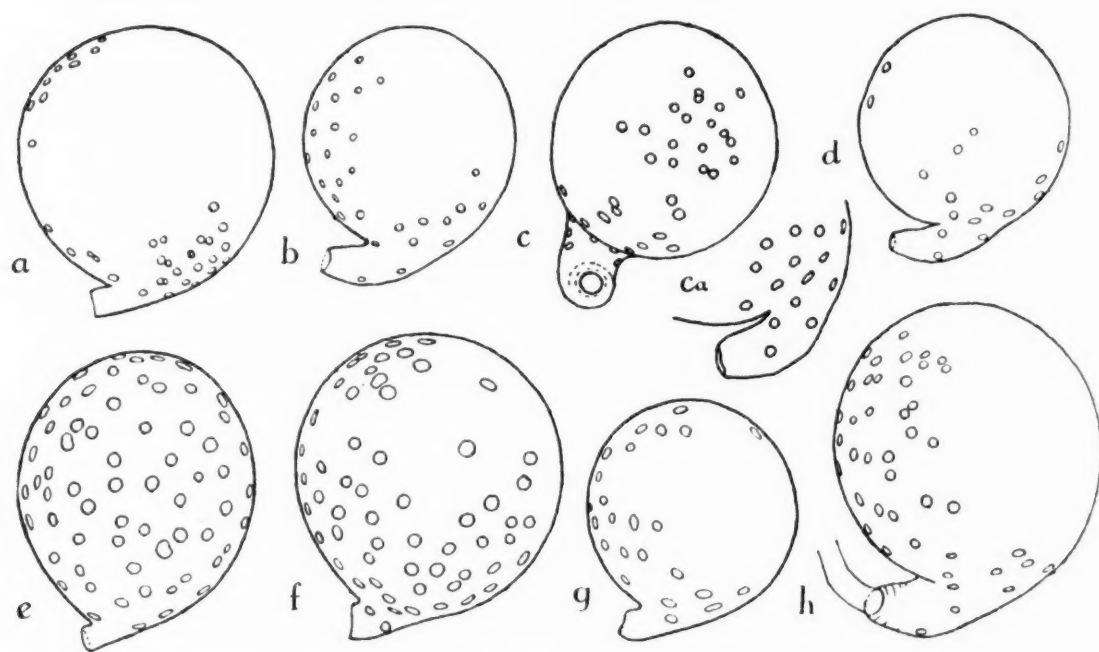


FIG. 3. Spermathecae of *a*—*Anopheles costalis*, Theo.; *b*—*A. marshalli*, Theo.; *c* and *ca*—*A. funestus*, Giles; *d*—*A. nili*, Theo.; *e*—*A. mauritanus*, Grp.; *f*—*A. pharoensis*, Theo.; *g*—*A. squamosus*, Theo., and *h*—*A. rufipes*, Gough.  $\times c. 250$ .

distribution of pale spots much as in *A. costalis*; the chitinised portion of the duct longer, about  $18\mu$ , somewhat curved and slightly constricted at its end.

*A. funestus*, Giles. Eight specimens. Generally similar to *A. costalis*. Cerci rather more slender; length varying from  $95\mu$  to  $120\mu$ , average  $108\mu$ , breadth from  $27\mu$  to  $36\mu$ , average  $32\mu$ . Spermatheca (fig. 3 *c* and *ca*) varying in length from  $91\mu$  to  $105\mu$ , average  $98\mu$ , and in breadth from  $84\mu$  to  $100\mu$ , average  $93\mu$ ; pale spots somewhat larger, but arranged much as in *A. costalis*;



chitinised portion of the duct long, varying from  $34\mu$  to  $47\mu$ , average  $40\mu$ ; curved and slightly constricted at the end.

*A. nili*, Theo. Two specimens. Generally similar to *A. costalis*. Cerci (fig. 2 *a*) smaller and narrower; length about  $105\mu$ , breadth about  $30\mu$ , and distal extremities more conical than rounded. Spermatheca (fig. 3 *d*)  $90\mu$  to  $108\mu$  in diameter; pale spots fewer and less distinct; chitinised portion of the duct long,  $30\mu$  to  $50\mu$ , somewhat curved and slightly constricted at its end.

*A. mauritianus*, Grp. Two specimens. Generally similar to *A. costalis*, but more highly chitinised. Cerci larger and of peculiar form (see fig. 2 *c*); length about  $180\mu$ , breadth in the middle about  $50\mu$ . Spermatheca (fig. 3 *e*) somewhat longer than broad, the diameters in two specimens measuring  $95\mu$  and  $85\mu$ , and  $114\mu$  and  $96\mu$  respectively; pale spots large, conspicuous, and scattered over the whole surface of the spermatheca; chitinised portion of the duct very short, similar to that of *A. costalis*, but shorter.

*A. pharoensis*, Theo. Two specimens. Generally similar to *A. costalis* but more highly chitinised. Cerci (fig. 2 *e*) very large, elongate-ovoid; length nearly  $300\mu$ , breadth about  $80\mu$ ; Spermatheca (fig. 3 *f*) highly chitinised, almost spherical, diameter about  $110\mu$ ; pale spots large, conspicuous, covering two-thirds of the surface; chitinised portion of the duct very short, similar to that of *A. mauritianus*.

*A. squamosus*, Theo. One specimen. Generally similar to *A. costalis* but more highly chitinised. Cerci (fig. 2 *b*) rather large, elongate-ovoid, with rounded ends; length about  $180\mu$ , breadth about  $50\mu$ . Spermatheca (fig. 3 *g*) similar to that of *A. costalis* but slightly smaller (diameter  $90\mu$ ) in the single specimen examined, and with the pale spots larger; chitinised portion of the duct as in *A. costalis*.

*A. rufipes*, Gough. Two specimens. Generally similar to *A. costalis* but more highly chitinised. Cerci similar to those of *A. funestus*; length  $130\mu$  to  $150\mu$ , breadth  $30\mu$  to  $50\mu$ . Spermatheca (fig. 3 *h*) sub-spherical, very highly chitinised; length  $130\mu$  to  $140\mu$ , breadth  $115\mu$  to  $118\mu$ ; pale spots as in *A. costalis* but rather larger; chitinised portion of the duct long, about  $35\mu$ , curved and slightly constricted at its end.

The eight species examined fall naturally into two groups: four



species, namely, *A. costalis*, *A. mauritanus*, *A. pharoensis* and *A. squamosus*, having only a very short portion, and four, namely, *A. marshalli*, *A. funestus*, *A. nili* and *A. rufipes*, having a considerably longer portion of the duct of the spermatheca chitinised. The species belonging to the first group can readily be distinguished by the arrangements of the pale spots on the spermatheca and by the form of the cerci. The species belonging to the second group can hardly be distinguished by these characters, although small differences between them may be noted, such as the large size of the spermatheca in *A. rufipes*, the feeble development of the pale spots and the rather narrow end to the cerci in *A. nili*, and the rather short and broad cerci in *A. marshalli*.

#### Tribe MEGARHININI

##### Genus *Toxorhynchites*

*T. brevipalpis*, Theo. One specimen. Posterior extremity of the abdomen blunt, cerci scarcely projecting; eighth segment not retracted. Sternite and tergite of the ninth segment highly chitinised, each appearing in a ventral view as a strip of chitin bent in the form of an arch with the opening directed anteriorly. Cerci (fig. 4) relatively small, with hatchet-shaped ends; length nearly

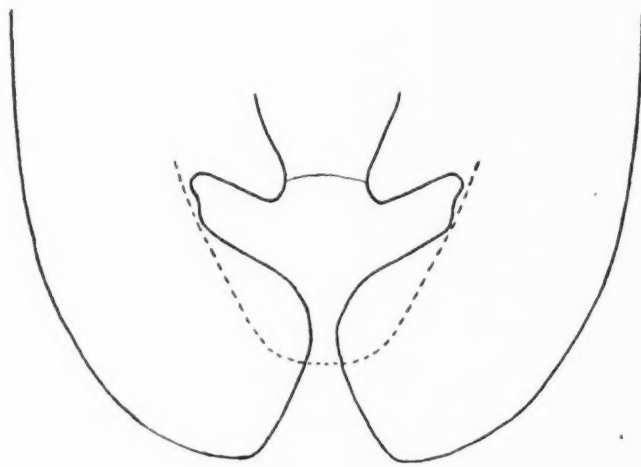


FIG. 4. *Toxorhynchites brevipalpis*, Theo., outlines of cerci and (dotted line) ventral process of the tenth segment, dorsal view.  $\times$  c. 185.

$300\mu$ , breadth  $114\mu$  at the widest part. Ventral process of the tenth segment bluntly conical, not emarginate, covered with bristles, none of which are, however, exceptionally long. Spermathecae three,

relatively small, highly chitinised, sub-spherical, the middle one rather larger than the other two, diameter about  $110\mu$ ; only the very commencement (about  $3\mu$ ) of the duct is chitinised.

Tribe CULICINI

Genus *Mucidus*

*M. scatophagoides*, Theo. (fig. 5 A). Two specimens. Posterior extremity of the abdomen tapering; the eighth segment sometimes completely withdrawn within the seventh. Sternite of the eighth

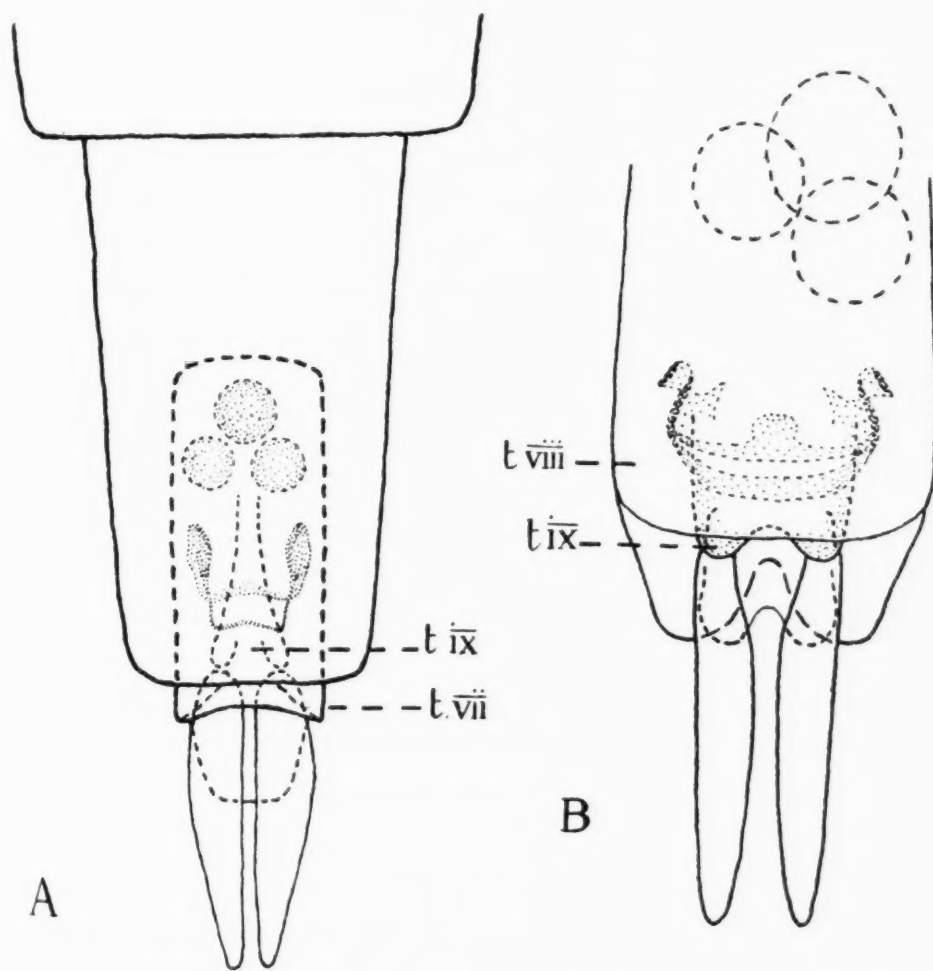


FIG. 5. Posterior extremity of abdomen of female, dorsal view. A—*Mucidus scatophagoides*, Theo.  $\times$  c. 92; B—*Banksinella lineatopennis*, Lud.  $\times$  c. 185 t.viii and t.ix—tergites of eighth and ninth segments.

segment deeply cleft posteriorly. Sternite of the ninth segment apparently reduced to narrow bands of chitin forming a double U-shaped loop. Cerci very long and leaf-like, tapering towards the

tips, length about  $350\mu$ , breadth about  $100\mu$ . At the bases of the cerci lies a long, straight plate of chitin, about  $250\mu$  in length, which is produced at its posterior end into two hairy lateral processes, runs directly anteriorly, and tapers gradually. This plate is apparently the ninth tergite. Ventral process on the tenth segment tongue-shaped, bearing a few stout setae, posterior margin broad, not emarginate. Spermathecae three, highly chitinated, sub-spherical or oblate-spheroidal, unequal, the middle one being slightly the largest. In the two specimens examined the diameters of the spermathecae were approximately  $65\mu$ ,  $76\mu$ ,  $65\mu$ , and  $74\mu$ ,  $93\mu$ ,  $74\mu$  respectively. The ducts of the spermathecae are chitinated only at their very commencement (about  $2\mu$ ).

#### Genus *Banksinella*

*B. lineatopennis*, Lud. (fig. 5 B). Three specimens. Genitalia of similar type to those of the genus *Stegomyia*. Posterior extremity of the abdomen tapering; the eighth segment may or may not be withdrawn within the seventh. Sternite of the eighth segment deeply notched posteriorly; tergite and sternite of the ninth segment relatively well developed. Cerci long, leaf-like, tapering towards their tips, somewhat similar to those of *Mucidus scatophagoides*; length about  $220\mu$ , breadth about  $60\mu$ . Ventral process of the tenth segment deeply notched in the middle line posteriorly. Spermathecae three, highly chitinated, sub-spherical (the length being slightly greater than the breadth), and unequal. The right and left spermathecae are about the same size, their diameters being about  $60\mu$ ; the middle one is larger, diameter about  $80\mu$ . The ducts of the spermathecae are chitinated for only a very short distance ( $1\mu$  to  $2\mu$ ) at their commencements.

#### Genus *Stegomyia*

Eight species were examined, all of which possess genitalia of a similar form; indeed, so close were the resemblances, that we were in most cases unable to detect differential points, and such differences as were noted appeared to be of only minor importance.

*S. fasciata*, F. (fig. 6). Ten specimens. Posterior extremity of the abdomen tapering slightly. Eighth segment not, or but slightly withdrawn within the seventh; sternite projecting beyond the

tergite and deeply notched in the middle line posteriorly. Tergite of the ninth segment shield-shaped, the posterior margin produced laterally into conical processes, which are well chitinised and armed with several stout setae. Sternite of the ninth segment represented by a narrow posterior strip of chitin which is arched so that its

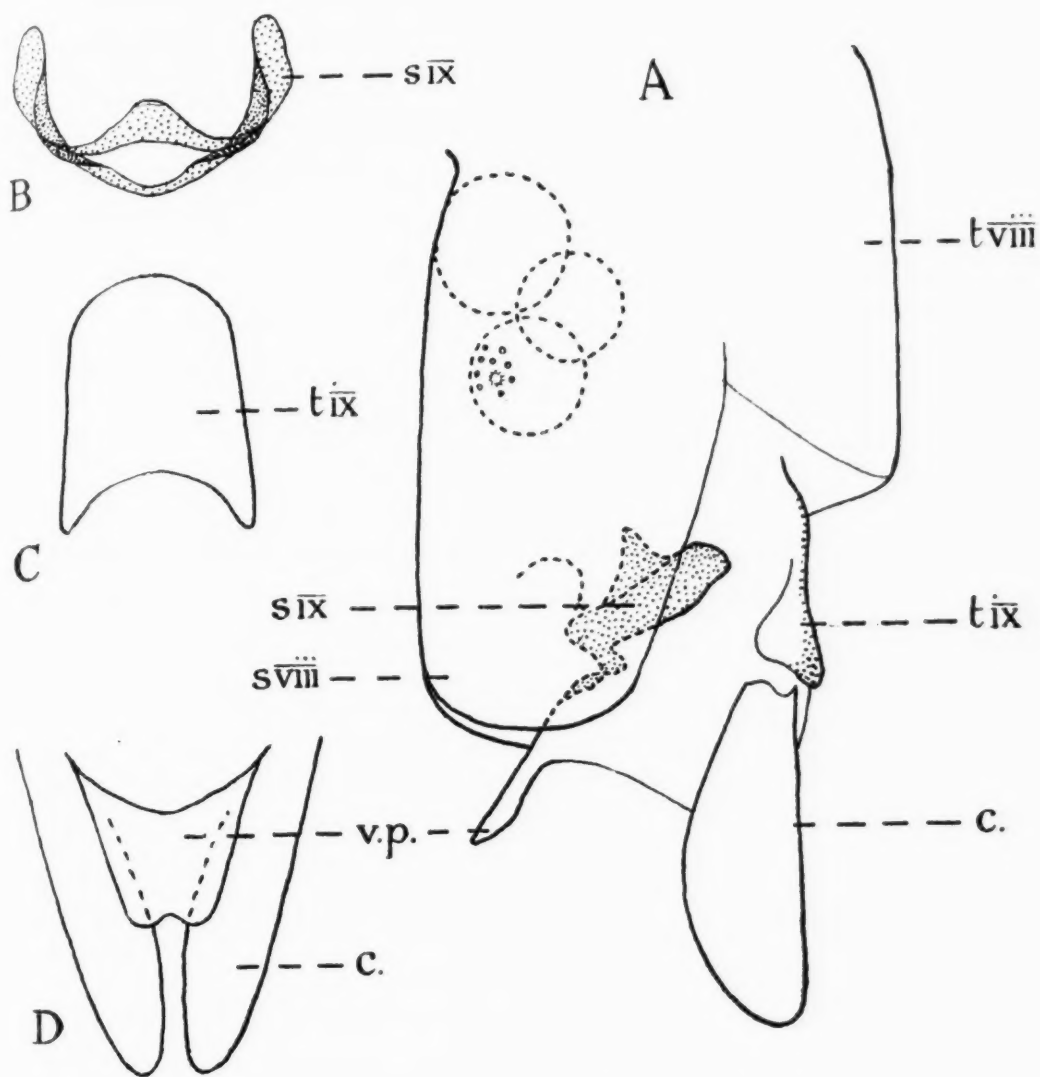


FIG. 6. *Stegomyia fasciata*, F. A—posterior extremity of body, lateral view; B—ninth sternite, ventral view; C—ninth tergite, dorsal view; D—cerci and ventral process of tenth segment, ventral view.  $\times$  c. 185.  $t̄$  viii and  $t̄$  ix—tergites of eighth and ninth segments;  $s̄$  viii and  $s̄$  ix—sternites of eighth and ninth segments; c—cerci; v.p.—ventral process of the tenth segment.

concavity is anterior and is expanded laterally, and a more delicate bar of chitin in front of it, which is broadest in the middle line. Cerci prominent, short and broad, hollowed out on their inner aspects, and inserted obliquely, that is with their broad surfaces converging dorsally; in the ten specimens measured the length

ranged from  $152\mu$  to  $198\mu$ , average  $183\mu$ , and the breadth from  $77\mu$  to  $103\mu$ , average  $85\mu$ . Ventral process of the tenth segment (fig. 7 *a*) with a moderately well developed notch in the middle of its posterior border, and bearing on each side several (about eight) long setae. Spermathecae three, highly chitinated, sub-spherical, the length being usually slightly greater than the breadth. The spermathecae are unequal and somewhat variable in size; the right and left ones are approximately the same size and in the ten specimens measured ranged in length from  $61\mu$  to  $80\mu$ , average  $69\mu$ , and in breadth from  $56\mu$  to  $76\mu$ , average  $63\mu$ ; the middle one is larger and ranged in length from  $84\mu$  to  $95\mu$ , average  $91\mu$ , and in breadth from  $70\mu$  to  $91\mu$ , average  $82\mu$ . A few pale spots similar to those in *Anopheles costalis* are sometimes present round the base. The ducts of the spermathecae are scarcely at all chitinated, at most for a distance of  $2\mu$  to  $4\mu$  at their commencement.

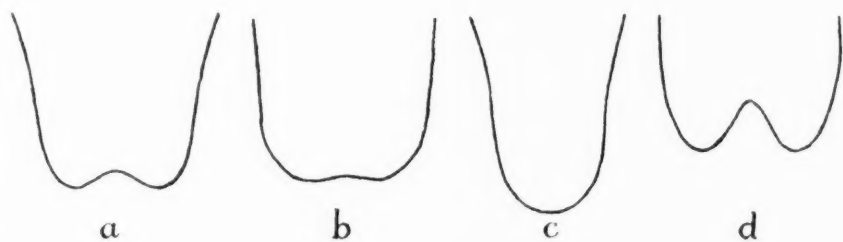


FIG. 7. Ventral process of the tenth abdominal segment, ventral view, of *a*—*Stegomyia fasciata*, F.; *b*—*S. unilineata*, Theo.; *c*—*S. vittata*, Bigot., and *d*—*Ochlerotatus albocephalus*, Theo.  $\times$  c. 250.

*S. apicoargentea*, Theo. One specimen. Very closely resembling *S. fasciata*. In the single specimen examined the only differences noted were that the setae on the posterior angles of the ninth tergite appeared to be longer, that the ventral process of the tenth segment was less deeply notched, and that the spermathecae were rather large, measuring  $84\mu$  by  $80\mu$ ,  $110\mu$  by  $91\mu$ , and  $80\mu$  by  $76\mu$ . These slight differences may be merely variations, and without confirmation from more materials are insufficient to distinguish the species.

*S. dendrophila*, Edw. Two specimens. Apparently indistinguishable from *S. fasciata*.

*S. luteocephala*, Newst. Four specimens. Very similar to *S. fasciata*, the only difference noted being the absence of the notch in the posterior border of the ventral process of the tenth segment.



*S. metallica*, Edw. One specimen. Apparently indistinguishable from *S. fasciata*, but notch in posterior border of the ventral process of the tenth segment shallow, as in *S. unilineata*.

*S. simpsoni*, Theo. One specimen. Apparently indistinguishable from *S. fasciata*, but the notch in the posterior border of the ventral process of the tenth segment is very shallow in the single specimen examined.

*S. unilineata*, Theo. Six specimens. Apparently indistinguishable from *S. fasciata*, but the notch in the posterior border of the ventral process of the tenth segment very shallow (fig. 7 *b*), as in *S. simpsoni* but cerci shorter.

*S. vittata*, Bigot. Three specimens. Very similar to *S. fasciata*, but the ventral process of the tenth segment long, tongue-like, without a notch (fig. 7 *c*).

#### Genus *Ochlerotatus*

Five species were examined; in all of them the genitalia were somewhat of the same type as those of species of *Stegomyia*. Two of the species, however, showed a remarkable divergence, inasmuch as they possessed only a single, large, spermatheca.

*O. albocephalus*, Theo. Two specimens. Similar to *S. fasciata*, but eighth segment usually more or less retracted within the seventh, eighth sternite more widely notched, cerci rather longer and narrower, length about  $190\mu$ , breadth about  $63\mu$ , ninth tergite smaller, less highly chitinised, notch in the posterior border of the ventral process of the tenth segment much deeper (fig. 7 *d*), and chitinised portion of the ducts of the three spermathecae a little longer, about  $4\mu$  to  $7\mu$ .

*O. apicoannulatus*, Edw. One specimen. Similar to *O. albocephalus*, but cerci relatively shorter and broader, length  $134\mu$ , breadth  $65\mu$  in the single specimen examined, and spermathecae (especially the middle one) a little larger but still well within the range of variation found in *S. fasciata*.

*O. domesticus*, Theo. One specimen. Similar to *O. albocephalus*, but eighth sternite more deeply notched, cerci longer, length about  $280\mu$ , breadth about  $68\mu$ , and notch in the ventral process of the tenth segment less deep.

*O. irritans*, Theo. (fig. 8). Seven specimens. General characters similar to those of *O. albocephalus*. Eighth segment usually partially withdrawn within the seventh, but capable of complete protrusion, disclosing a wide membranous junction between the two segments. Eighth sternite slightly longer than the tergite, notch rather shallow. Cerci of usual form: average length  $186\mu$ , average breadth  $83\mu$ .

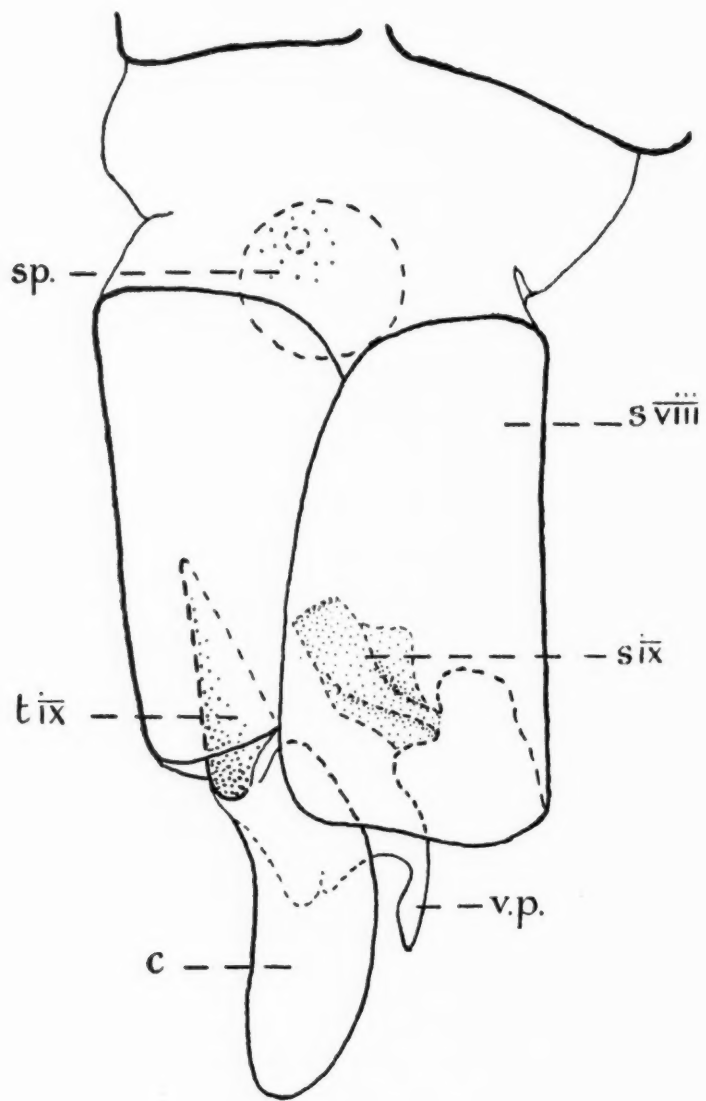


FIG. 8. *Ochlerotatus irritans*, Theo., posterior extremity of abdomen of female, lateral view.  $\times$  c. 185.

Ventral process of the tenth segment deeply notched posteriorly, as in *O. albocephalus*. Spermatheca single, large, sub-spherical; average length  $93\mu$ , breadth  $89\mu$ . There are at the base a number of pale spots, as in *A. costalis*. Duct chitinised for only a short distance, about  $5\mu$ , at its commencement.

*O. punctothoracis*, Theo. One specimen. Apparently almost indistinguishable from *O. irritans*, but the cerci are rather smaller in the single specimen examined and are more pointed at their tips.

#### Genus *Mansonioides*

*M. africanus*, Theo. (figs. 9 and 10). Eight specimens. Posterior extremity of the abdomen bluntly conical. Eighth segment may be partially retracted within the seventh, tergite narrow, posterior margin armed with a row of strong recurved teeth arranged as shown in the figure (fig. 9), the middle group composed of seven, or more commonly nine teeth, the central one being the longest, the two lateral groups of from five to seven teeth; sternite much longer than the tergite, and prolonged on each side posteriorly as a wide flap which is deeply notched. Ninth segment much reduced, the tergite represented by a narrow arch of chitin, and the sternite by the usual transverse sclerites, which are rather poorly developed. Cerci rather short with their narrowest diameter directed dorso-ventrally, slightly concave dorsally, and ending in a rather sharp tip; length variable, average about  $192\mu$ , middle lateral breadth about  $90\mu$ . Ventral process of the tenth segment very deeply cleft in the middle line posteriorly. Spermathecae three, two large and very highly chitinised, and one very small and feebly chitinised. The two large spermathecae are sub-equal and sub-spherical; average length  $137\mu$ , breadth  $125\mu$ . They have a slight bulge near the point of origin of the duct (fig. 13 A), and there are numerous small pale spots at the base. The ducts are chitinised for only a short distance (about  $10\mu$ ) at the commencement. The small spermatheca is sub-spherical, length about  $29\mu$ , breadth about  $28\mu$ , it is usually feebly and incompletely chitinised, its base being membranous, and is difficult to find if the abdomen is incompletely cleared, and may for this reason be overlooked. Its duct joins the duct of one of the large spermathecae, so that it clearly represents an ill-developed lateral spermatheca.

*M. uniformis*, Theo. Five specimens. As in *M. africanus*, but the lateral flaps of the eighth sternite are not notched. In the five specimens examined it was also noted that the teeth in the lateral groups on the dorsum of the eighth segment were rather more variable and numbered from three to six, and that the small

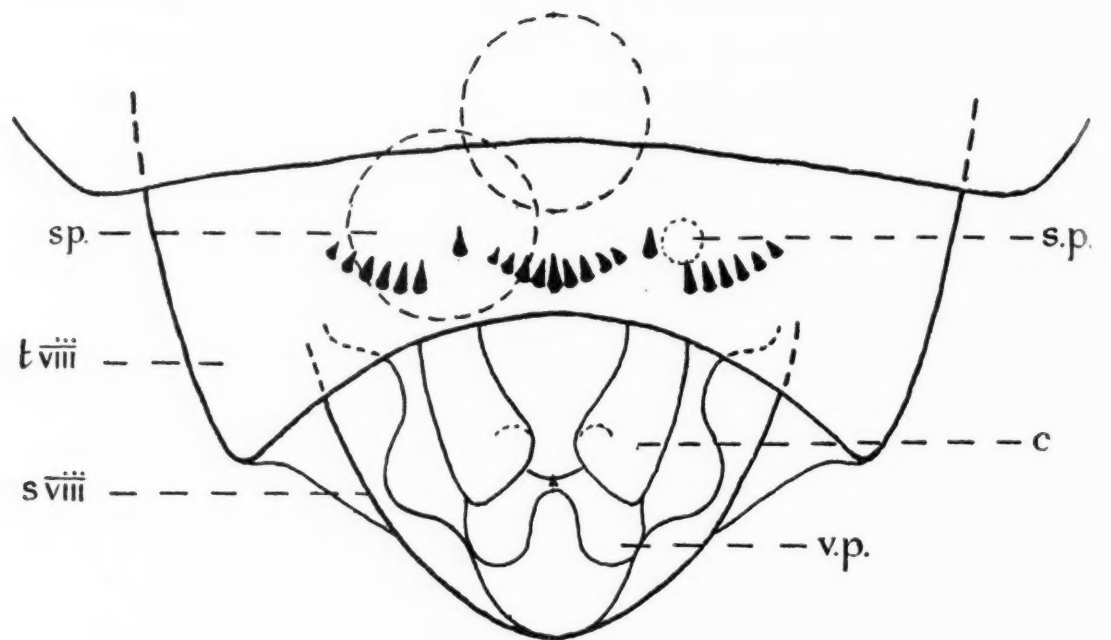


FIG. 9. *Mansonoides africanus*, Theo., posterior extremity of abdomen of female, dorsal view.  $\times$  c. 150.

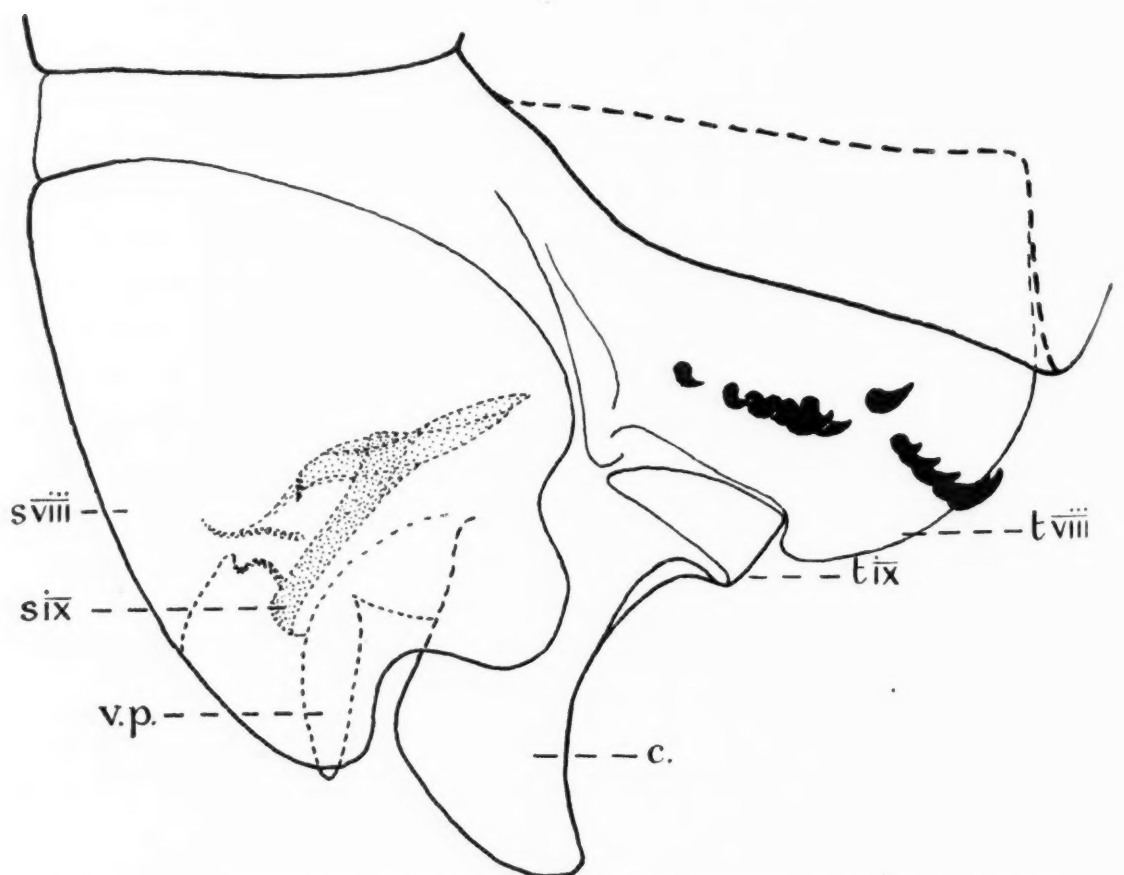


FIG. 10. *Mansonoides africanus*, Theo., posterior extremity of abdomen of female, lateral view.  $\times$  c. 150.

spermatheca was sometimes rather large, in one instance measuring  $65\mu$  by  $53\mu$ ; these latter differences are probably not specific.

Genus *Aedomyia*

*Aedo. africana*, Nev.-Lem. (figs. 11 and 12). Two specimens. Similar to *Anopheles*. Posterior extremity of the abdomen blunt,

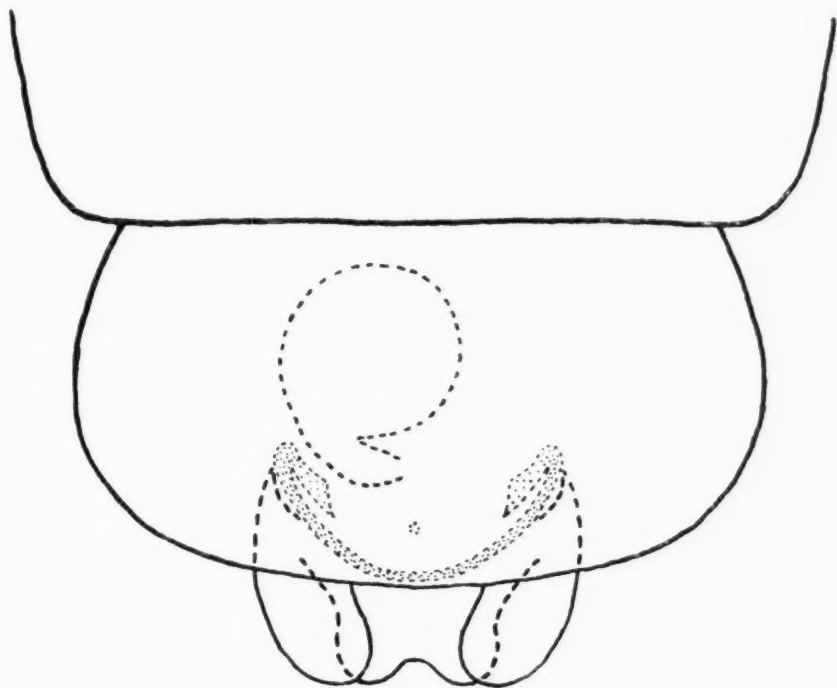


FIG. 11. *Aedomyia africanus*, N. L., posterior extremity of abdomen of female, dorsal view.  $\times$  c. 185.

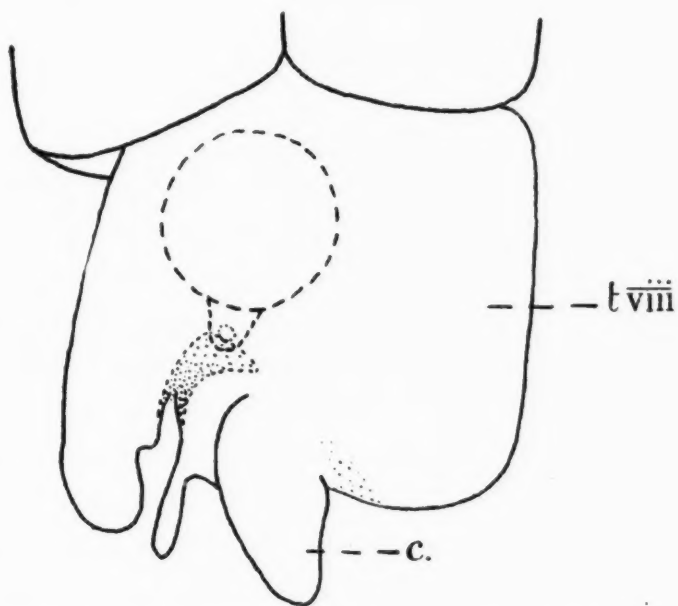


FIG. 12. *Aedomyia africanus*, N. L., posterior extremity of abdomen of female, lateral view.  $\times$  c. 185.



cerci not prominent. Eighth segment not withdrawn within the seventh; sternite with a shallow notch. Ninth segment much reduced, feebly chitinised. Cerci short and broad, with blunt, rounded ends; length  $118\mu$ , breadth  $65\mu$ . Ventral process of the tenth segment short and broad with a wide notch in its posterior border, bearing on each side several stout setae. Spermatheca single, very highly chitinised, resembling that of *A. junestus*; length  $106\mu$ , breadth  $97\mu$ , length of the chitinised portion of the duct  $45\mu$ ; the whole spermatheca is sparsely dotted with pale spots, which, however, are small and are most numerous at the base.

#### Genus *Taeniorhynchus*

*T. aurites*, Theo. (fig. 13, B to D). One specimen. In some respects similar to *Mansonioides*. Eighth segment only slightly withdrawn from the seventh and capable of complete protrusion; sternite long, not notched, tergite short, without teeth. Ninth segment reduced, much as in *Mansonioides*. Cerci (fig. 13 B and C)

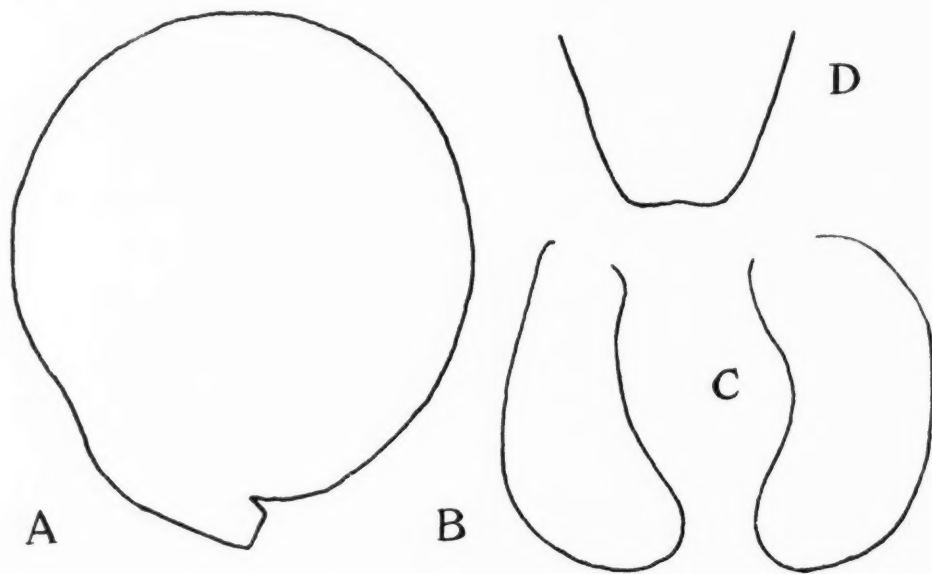


FIG. 13. A—*Mansonioides africanus*, Theo., spermatheca;  $\times$  c. 375. B—*Taeniorhynchus aurites*, Theo., outline of one of the cerci in ventral view, and C—in lateral view; and of D—ventral process of the tenth segment;  $\times$  c. 185.

curved dorsally, short, broad, with rounded extremities; length  $182\mu$ , breadth  $80\mu$ . Ventral process of the tenth segment hardly at all notched in the middle line posteriorly (fig. 13 D). Spermathecae three, rather poorly chitinised, sub-spherical, large, unequal; in the single specimen examined they were not fully expanded, but, so far

as could be judged, their diameters were respectively about  $100\mu$ ,  $115\mu$ , and  $122\mu$ . A short portion of the commencement of the ducts is feebly chitinised.

#### Genus *Culex*

Twelve species were examined, all of which possess genitalia of a very similar form, so that points of distinction, when found, are but slight and sometimes difficult to detect. In all the species the posterior extremity of the abdomen is blunt, the eighth sternite notched posteriorly, and the cerci relatively small, short, broad, and obliquely set. On the lining membrane, just below the posterior border of the eighth sternite, is a tuft-like group of more or less stout setae. The U-shaped structure surrounding the vulva is well chitinised. The ventral process of the tenth segment is short, occasionally notched, and not very hairy. There are three spermathecae, which are usually oval, and their ducts are chitinised for only a short distance. Points of distinction between species appeared to be furnished by all the above structures. It may be mentioned here that the species belonging to the Genera *Culiciomyia*, *Eumelanomyia*, and *Micraedes*, which we have examined, also possess genitalia of the same type.

*C. fatigans*, Wied. (figs. 14 and 15). Ten specimens. Posterior extremity of the abdomen blunt, cerci not very prominent. Eighth segment not withdrawn within the seventh, sternite prolonged posteriorly beyond the tergite, and shallowly notched. From the middle of the membrane lining the posterior border of the eighth sternite arises a tuft-like group of about ten rather stout setae. Ninth segment, as usual, much reduced; tergite a narrow strip, broadest laterally and rather feebly chitinised. Ventrally there is a horseshoe-shaped strip of chitin, open anteriorly, enclosing the vulva, and just posterior to it a wider arch of chitin, from the lateral portions of which rather broad but feebly chitinised plates project inwards. Cerci set slightly obliquely, concave internally, short and broad, with truncated ends; in the ten specimens measured, length from  $137\mu$  to  $170\mu$ , average  $150\mu$ , and breadth from  $80\mu$  to  $91\mu$ , average  $85\mu$ . Ventral process of the tenth segment (fig. 17 *b*) without a notch, bearing at its apex a few stout setae, and on the ventral aspect a few (two or three on each side) smaller ones.

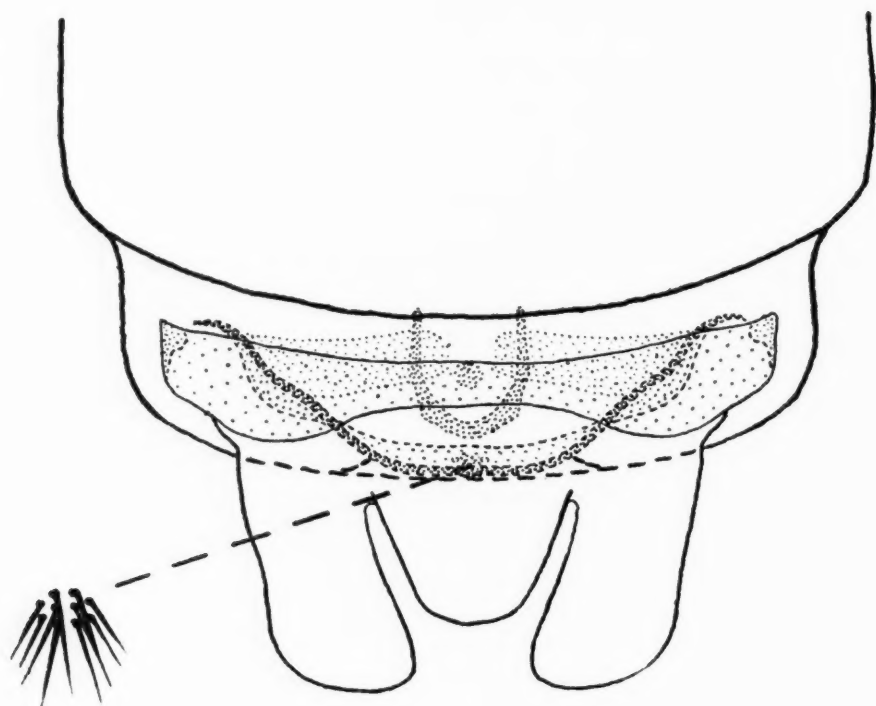


FIG. 14. *Culex fatigans*, Wied., posterior extremity of abdomen of female, dorsal view.  
 X c. 185.

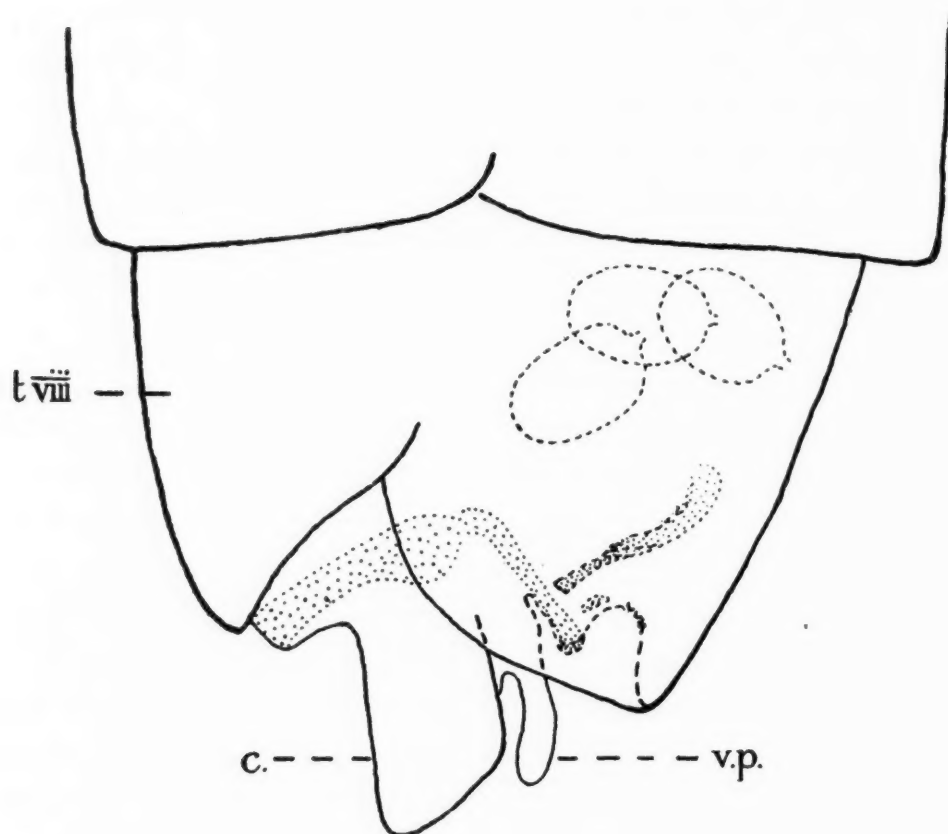


FIG. 15. *Culex fatigans*, Wied., posterior extremity of abdomen of female, lateral view.  
 X c. 185.

Spermathecae (fig. 16 *c*) three, very highly chitinised, sub-equal, the middle one being slightly the largest; in the ten specimens measured the length ranged from  $72\mu$  to  $99\mu$ , average  $84\mu$ , and the breadth from  $55\mu$  to  $76\mu$ , average  $63\mu$ . They are somewhat variable in shape but are usually oval, sometimes almost sub-spherical, and commonly the base is rather broad and the apex narrowed so that they resemble a bee-hive. At the base there are a few 'pale spots.' The chitinised portion of the ducts is short, conical, and in the specimens measured ranged in length from  $6\mu$  to  $11\mu$ , average  $8\mu$ .

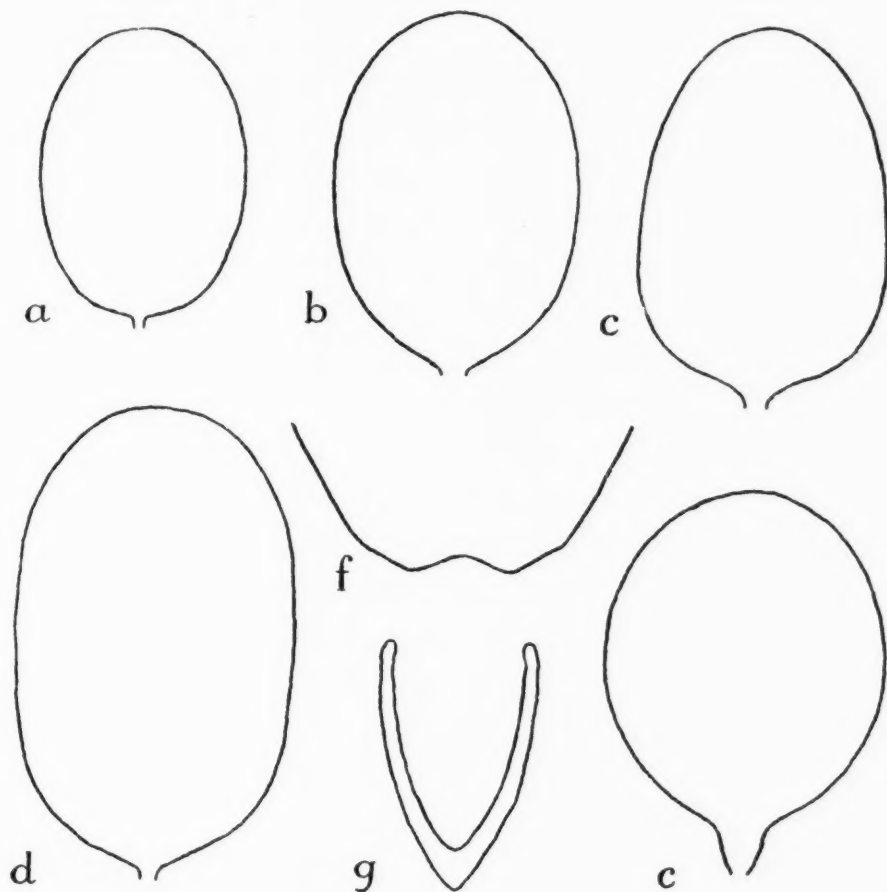


FIG. 16. Outlines of spermathecae of *a*—*Culex insignis*, Cart.; *b*—*C. annulioris*, Theo.; *c*—*C. fatigans*, Wied.; *d*—*C. consimilis*, Newst., and *e*—*C. duttoni*, Theo.; and of *f*—the ventral process of the tenth segment, and *g*—the chitinous hoop round the vulva, of *C. annulioris*, Theo. All  $\times c. 375$ .

*C. annulioris*, Theo. One specimen. As in *C. fatigans*, but the inner chitinous bar enveloping the vulva (fig. 16 *g*) is narrower, almost V-shaped, and the ventral process of the tenth segment is shallowly notched and bears four or five small setae on each side on its ventral aspect (fig. 17 *d*). The spermathecae (fig. 16 *b*) are

highly chitinised, sub-equal, oval; length  $99\mu$ , breadth  $68\mu$ , the chitinised portion of the ducts very short, about  $2\mu$ .

*C. consimilis*, Newst. One specimen. As in *C. fatigans*, but the spermathecae are rather larger (fig. 16d). They are highly chitinised, a rather long, oval shape, and not narrowed at the apex; in the specimen examined the middle one measured  $137\mu$  in length by  $84\mu$  in breadth, and the chitinised portion of its duct was about  $7\mu$  long, and in the other two spermathecae the corresponding

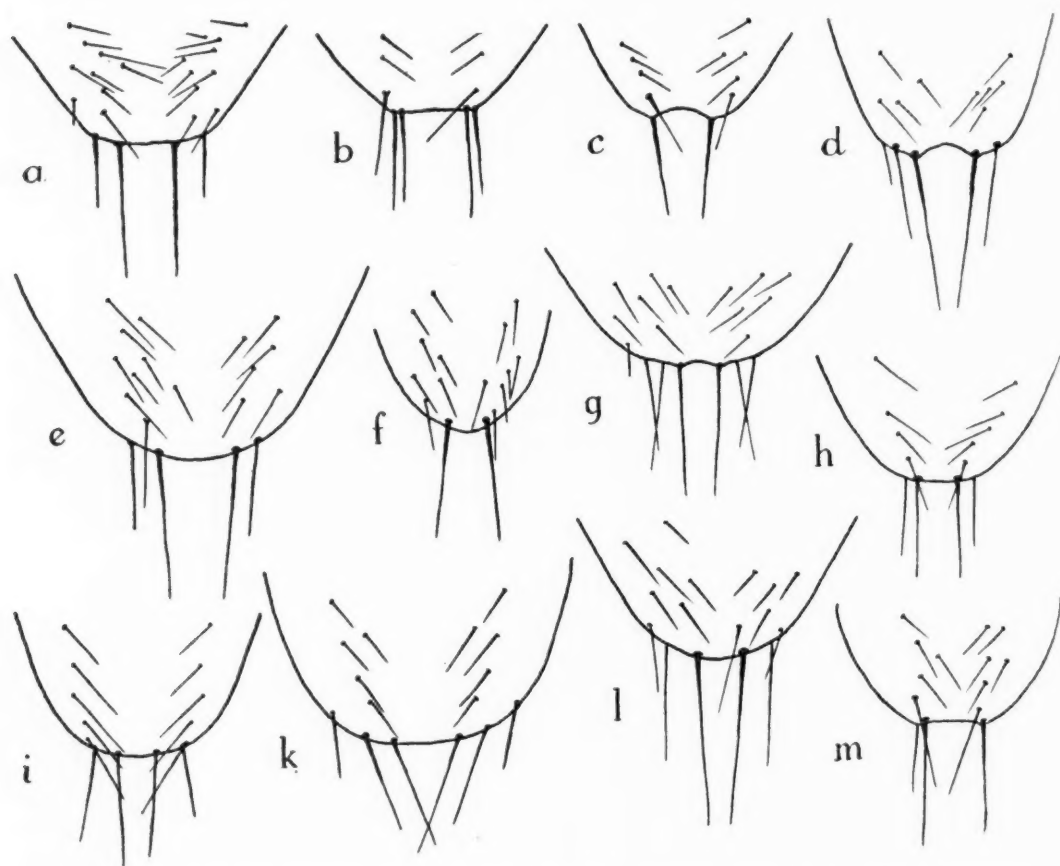


FIG. 17. Ventral process of the tenth segment, ventral view of a—*Culex consimilis*; b—*C. fatigans*; c—*C. decens*; d—*C. annulioris*; e—*C. duttoni*; f—*C. insignis*; g—*C. pruina*; h—*C. quasigelandus*; i—*C. rima*; k—*C. thalassius*; l—*C. tigripes* var. *fuscus*; and m—*C. tritaeniorhynchus*.  $\times 250$ .

measurements were  $129\mu$ ,  $80\mu$ , and  $4\mu$  respectively. The ventral process of the tenth segment (fig. 17a) is more hairy than in *C. fatigans*, and bears about nine small setae on each side on its ventral aspect.

*C. decens*, Theo. Twelve specimens. Similar to *C. fatigans*, but in the specimens examined the cerci were rather more prominent, length about  $160\mu$ , breadth about  $70\mu$ ; the tuft of setae on the lining



membrane of the posterior end of the eighth sternite usually rather larger, composed of about a dozen setae; chitinous loop enclosing the vulva not so wide, more U-shaped; the ventral process of the tenth segment (fig. 17 *c*) with a shallow notch, small setae on ventral aspect rather variable, from three to nine on each side; and the spermathecae not so highly chitinised, a little larger, average length  $90\mu$ , breadth  $68\mu$ .

*C. duttoni*, Theo. Two specimens. Generally similar to *C. fatigans*. Tuft of setae on the lining membrane of the eighth sternite rather larger, composed of twelve setae; and ventral process of tenth segment (fig. 17 *e*) more hairy, bearing about six to nine small setae on each side on its ventral aspect. Cerci short and broad; length about  $175\mu$ , breadth about  $115\mu$ . Spermathecae (fig. 16 *e*) very highly chitinised, sub-spherical, the middle one slightly the largest; average length about  $85\mu$ , average breadth about  $77\mu$ , the chitinised portions of the ducts conical, rather long, average length about  $15\mu$ .

*C. insignis*, Carter. One specimen. Generally similar to *C. fatigans*. Eighth sternite more deeply notched posteriorly; and ventral process of the tenth segment (fig. 17 *f*) more conical and more hairy, bearing about six or seven small setae on each side on its ventral aspect. Chitinised bar encircling the vulva rather strong and thick, and omega-shaped. Tuft of setae on the lining membrane of the eighth sternite rather small, composed of eight setae. Cerci rather small and curved dorsally; length  $114\mu$ , breadth  $57\mu$ . Spermathecae (fig. 16 *a*) very highly chitinised, oval, the middle one measuring about  $95\mu$  by  $68\mu$ , and the lateral ones  $84\mu$  by  $57\mu$ ; the chitinised portion of the ducts is short ( $5\mu$  to  $6\mu$ ) and narrow ( $4\mu$ ).

*C. pruina*, Theo. One specimen. Apparently almost indistinguishable from *C. fatigans*, but in the single specimen examined the chitinous loop enclosing the vulva was rather narrower, as in *C. annulioris*, and the spermathecae were not so heavily chitinised, more regularly oval, and longer, having a length of about  $105\mu$ , breadth about  $80\mu$ , and the chitinised portion of the ducts about  $6\mu$ . Small setae on the ventral aspect of the ventral process of the tenth segment (fig. 17 *g*) rather more numerous, about six or seven on each side.

*C. quasigelidus*, Theo. One specimen. As in *C. fatigans*, but in the single specimen examined the loop of chitin enclosing the vulva is more V-shaped, and the spermathecae are rather larger, the middle one measuring  $103\mu$  by  $72\mu$ , the lateral ones  $91\mu$  by  $69\mu$ , and the ducts being short,  $4\mu$  and  $2\mu$  respectively.

*C. rima*, Theo. One specimen. Closely resembling *C. insignis*. In the single specimen examined the cerci were small and curved dorsally, as in *C. insignis*, and the apices of the spermathecae were broad and not narrowed as they often are in *C. fatigans*. The eighth sternite also appeared to be more deeply notched than in *C. fatigans*, and the ventral process of the tenth segment (fig. 17 i) more hairy, bearing a row of about five small setae on each side on the ventral aspect.

*C. thalassius*, Theo. Seven specimens. Similar to *C. fatigans*, but loop of chitin enclosing the vulva rather narrower posteriorly, and spermathecae more regularly oval, and in some specimens a little larger ( $103\mu$  by  $68\mu$  in one). Small setae on the ventral aspect of the ventral process of the tenth segment (fig. 17 k) rather more numerous, about five on each side.

*C. tigris*, Grp., var. *fuscus*, Theo. Three specimens. Very highly chitinised. Generally similar to *C. fatigans*, but larger. Cerci about  $180\mu$  by  $105\mu$ . Spermathecae very highly chitinised, shaped as in *C. fatigans*, the middle one the larger, about  $110\mu$  by  $85\mu$ , the lateral ones about  $97\mu$  by  $76\mu$ ; the chitinised portion of the ducts is about  $8\mu$  long. The tuft of setae on the lining membrane of the eighth sternite is rather larger than in *C. fatigans*. The ventral process of the tenth segment (fig. 17 l) is not notched, and is rather more conical and hairy than in *C. fatigans*, there being about six small setae on each side on the ventral aspect.

*C. tritaeniorhynchus*, Giles. Two specimens. Apparently indistinguishable from *C. fatigans*, but the spermathecae are, perhaps, a little more regularly oval, and the ventral process of the tenth segment (fig. 17 m) rather more hairy, having four or five small setae on each side on its ventral aspect.

The genitalia of the twelve species examined were so much alike that they could be distinguished, if at all, only by means of minute differences, which in some cases cannot be accepted as of specific value owing to the materials being insufficient to exclude the error

due to the natural range of variation. Judging solely from the specimens we have examined, however, points of distinction appeared to be present in the size of the cerci, the shape and size of the spermathecae, the shape of the chitinised hoop round the vulva, the shape of the ventral process of the tenth segment, and the number of small setae (not including the larger setae near the apex) on the ventral aspect of this process.

From all the other species examined *C. duttoni* is readily distinguished by the sub-spherical shape of the spermathecae and the relatively long chitinised portion of the ducts, and *C. consimilis* by the large size of its oval spermathecae and the relatively numerous small setae (about nine on each side) on the ventral aspect of the ventral process of the tenth segment. Two other species, *C. annulioris* and *C. decens*, may, perhaps, be separated by the fact that in them the ventral process of the tenth segment is notched; and *C. tigripes* may be recognised by its size. Other points that may be of systematic value, such as the small size of the cerci in *C. rima* and *C. insignis*, and the scantiness of the hairs on the ventral process of the tenth segment in *C. fatigans* and *C. quasi-gelidus*, can be confirmed only by further experience.

#### Genus *Culiciomyia*

*C. nebulosa*, Theo. Five specimens. Genitalia of the same type as in the genus *Culex*, and very similar to those of *C. fatigans*, from which they appeared to differ only in having shorter cerci (about  $115\mu$  by  $72\mu$ ), and in the ventral process of the tenth segment being shallowly notched and slightly more hairy, having about five small setae on each side on the ventral aspect. The spermathecae are also rather larger and less highly chitinised; the average measurements of the middle one being, length  $99\mu$ , breadth  $69\mu$  (one specimen measuring  $110\mu$  by  $72\mu$ ), and the lateral ones, length  $85\mu$ , breadth  $63\mu$ ; the chitinised portions of the ducts measure about  $7\mu$  in length.

#### Genus *Eumelanomyia*

*E. inconspicua*, Theo. Three specimens. Genitalia of the same type as in the genus *Culex*. Posterior extremity of the abdomen blunt, cerci not prominent, seldom projecting beyond the

eighth sternite. Tuft on the lining membrane of the eighth sternite small, composed of eight not very strong setae. Ninth segment as in *C. fatigans*, but loop enclosing the vulva very feebly chitinised. Cerci small, broad, extremities directed inwards; length about  $105\mu$ , greatest lateral breadth about  $50\mu$ . Ventral process of the tenth segment not notched, bearing a few hairs, none of which are very strong. Spermathecae three, oval, rather feebly chitinised, the middle one slightly the largest; average length about  $88\mu$ , breadth  $65\mu$ , the chitinised portion of the ducts short, about  $7\mu$ .

#### Genus *Micraedes*

*M. inconspicuus*, Theo. One specimen. Genitalia of the *Culex* type. Posterior extremity of the abdomen blunt, cerci not prominent. Eighth segment not retracted within the seventh; sternite with a shallow notch posteriorly. Ninth segment reduced as usual. Ventrally there is a small U-shaped bar of chitin enclosing the vulva, and more posteriorly a second strip of chitin forming a transverse arch. From the lining of the posterior part of the eighth sternite, in the middle line, there projects backwards a tuft-like group of eight (four pairs) stout setae. Cerci short and broad, with truncated extremities; length  $76\mu$ , breadth  $45\mu$ . Ventral process of the tenth segment projecting a little beyond the cerci, broad, very slightly notched, and bearing on each side a few rather feebly chitinised setae, two of which, one apical and one slightly dorsal, are rather large. Spermathecae three, relatively large, moderately well chitinised, oval, and sub-equal; length about  $57\mu$ , breadth about  $46\mu$ . The chitinised commencements of the ducts are conical and rather long, about  $10\mu$ .

#### Genus *Mimomyia*

*M. splendens*, Theo. (fig. 18, A and B). Three specimens. Posterior extremity of the abdomen bluntly conical, cerci rather prominent. Eighth segment not withdrawn within the seventh. Ninth segment much as usual; no U-shaped loop of chitin enclosing the vulva. Cerci of the usual form, obliquely set, rather small, length about  $115\mu$ , lateral breadth about  $65\mu$ . Ventral process of the tenth segment reaching posteriorly as far as the cerci, broad, very



hairy, apically and ventrally, and deeply notched. Spermatheca single, highly chitinised, sub-spherical, and relatively very large, diameter about  $105\mu$  to  $115\mu$ ; there are numerous pale spots at the base, and almost no part of the duct is chitinised.

*M. mimomyiaformis*, Newst. Two specimens. Similar to *M. splendens*, but in the specimens examined the cerci were very short and broad, length  $95\mu$ , lateral breadth  $72\mu$ , and the ventral process of the tenth segment projected posteriorly beyond the cerci and was only feebly notched (fig. 18 c).

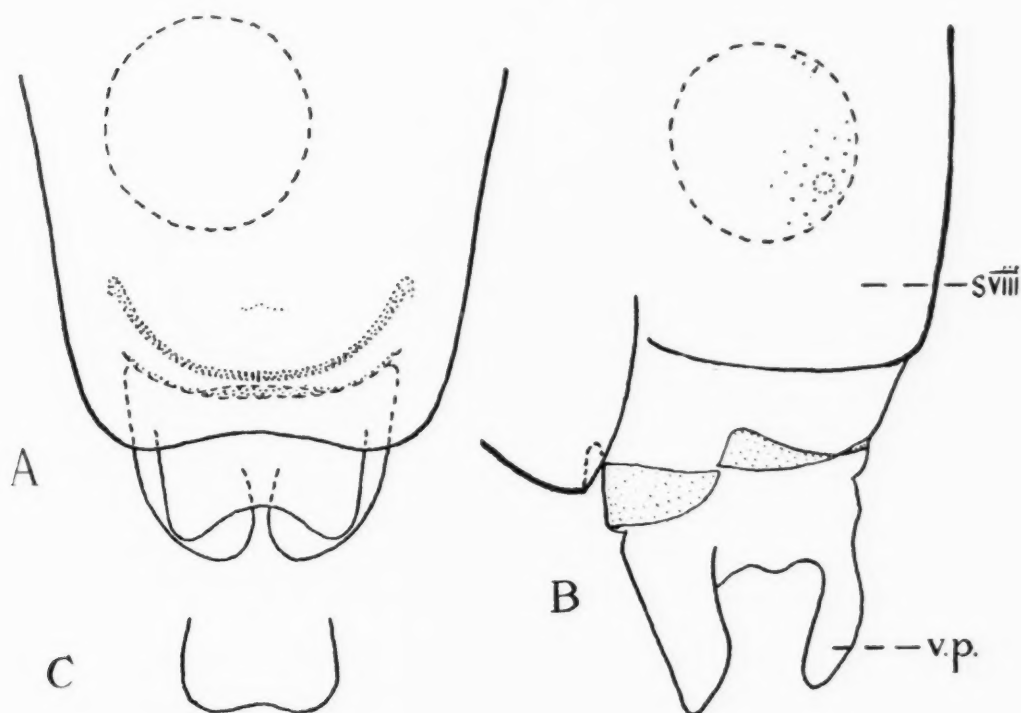


FIG. 18. *Mimomyia splendens*, Theo., posterior extremity of abdomen of female. A—ventral view; B—lateral view.  $\times$  c. 185. *Mimomyia mimomyiaformis*, Newst.; C—ventral process of the tenth segment, ventral view.  $\times$  c. 185.

*M. plumosa*, Theo. (fig. 19). One specimen. Genitalia unlike those of the two preceding species. Chitinisation of the ninth sternite rather strong, but there is no loop enclosing the vulva. Cerci obliquely set as usual, appearance varying greatly with the position: in a ventral view they are truncated, in a lateral view they are cone-shaped with a rather pointed extremity, and in sub-lateral view (the lateral aspect of the cerci) they are short and broad, about  $150\mu$  by  $115\mu$ , with their dorsal extremities prolonged into a



process. Ventral process of the tenth segment large, very hairy, deeply notched posteriorly (fig. 19 B). Spermathecae three, highly chitinised, sub-spherical to oval, the middle one the largest and measuring about  $148\mu$  by  $137\mu$ , the lateral ones smaller, about  $122\mu$  by  $106\mu$ ; practically no part of the ducts is chitinised.

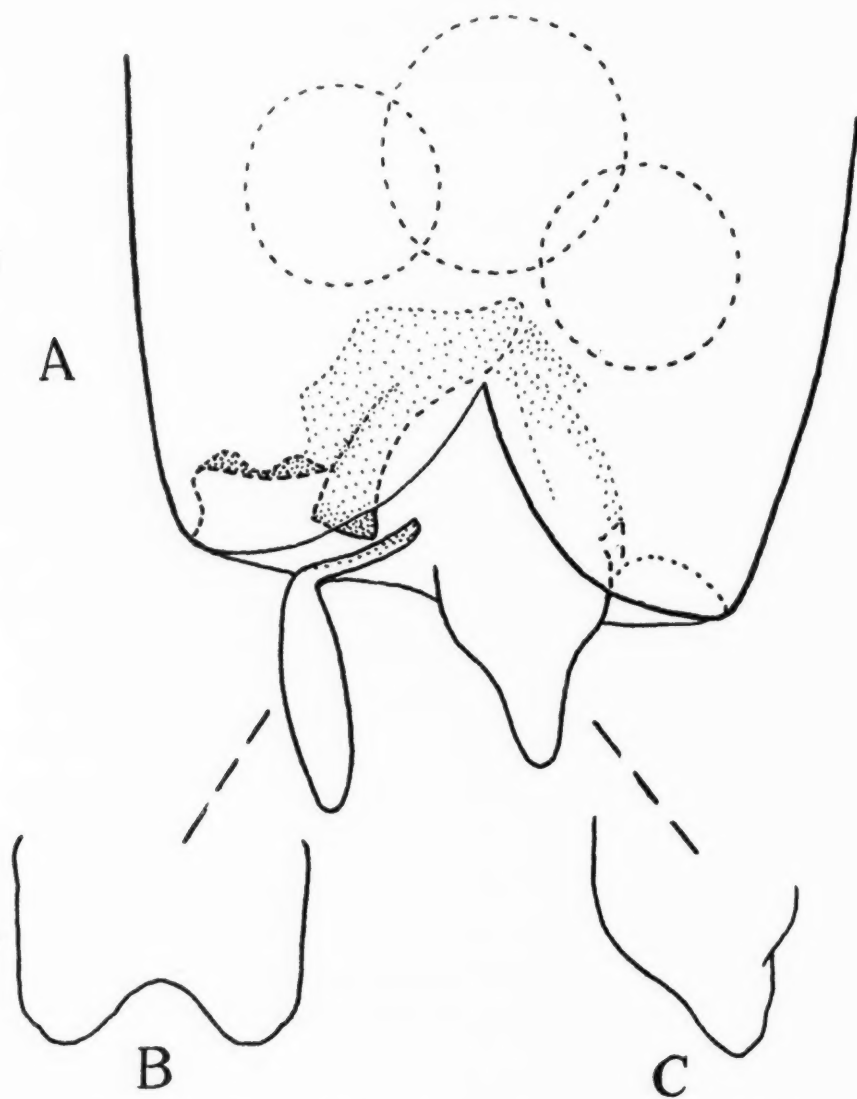


FIG. 19. *Mimomyia plumosa*, Theo. A—posterior extremity of abdomen of female, lateral view; B—ventral process of the tenth segment, ventral view; and C—one of the cerci, sub-lateral view.  $\times$  c. 185.

#### Genus *Uranotaenia*

*U. balfouri*, Theo. One specimen. Very small, posterior extremity of the abdomen bluntly conical, the terminal segments not so far retracted as usual. Cerci (fig. 20 B) very short, broad; length

about  $60\mu$ , breadth about  $45\mu$ . Ventral process of the tenth segment about as long as the cerci, broad, without a notch and bearing a few but no very large setae. Spermatheca single, sub-

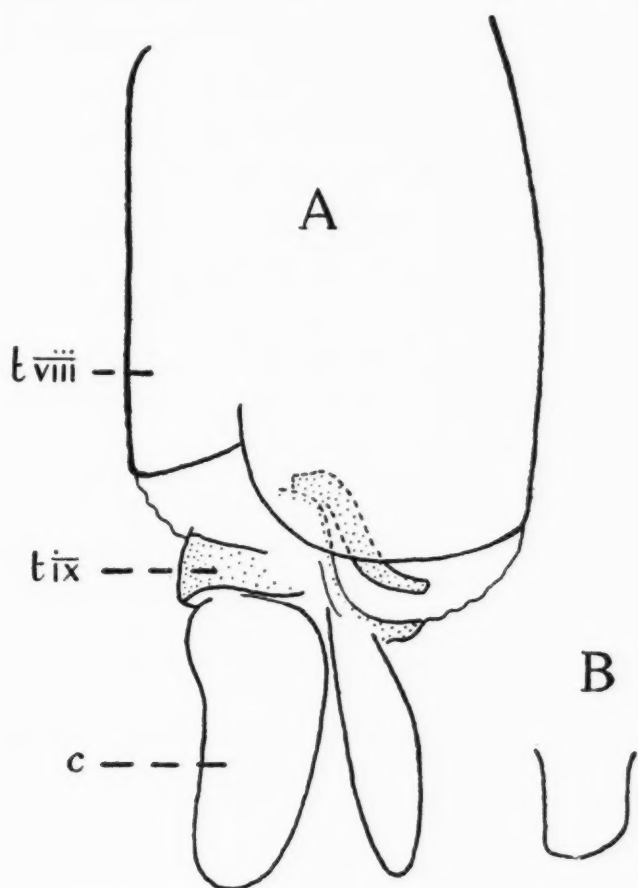


FIG. 20. A—*Uranotaenia annulata*, Theo., posterior extremity of abdomen of female, lateral view.  $\times$  c. 185. B—*Uranotaenia balfouri*, Theo., one of the cerci, lateral view.  $\times$  c. 185.

spherical, length about  $91\mu$ , breadth about  $84\mu$ ; only the very commencement of the duct is chitinised.

*U. annulata*, Theo. (fig. 20 A). One specimen. Generally similar to *U. balfouri*, but larger. Cerci rather long, with bluntish ends; length about  $170\mu$ , breadth about  $68\mu$ . Ventral process of the tenth segment nearly as long as the cerci, without a notch, bearing numerous setae, those at the apex being large. Spermatheca single, sub-spherical, length  $80\mu$ , breadth  $76\mu$ ; practically no part of the duct (which is long and narrow) is chitinised.

#### Tribe SABETHINI

##### Genus *Eretmopodites*

*E. chrysogaster*, Grah. (figs. 21 and 22). Six specimens. Very highly chitinised. Posterior extremity of the abdomen blunt, cerci

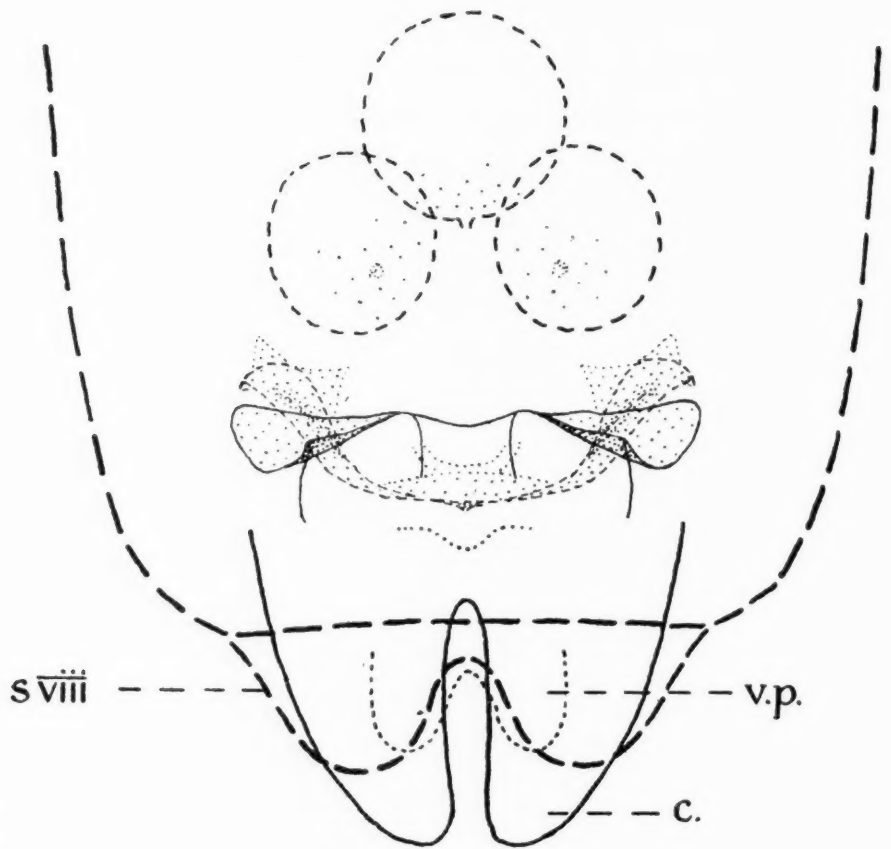


FIG. 21. *Eretmopodites chrysogaster*, Grah., posterior extremity of abdomen of female, dorsal view.  $\times$  c. 185;

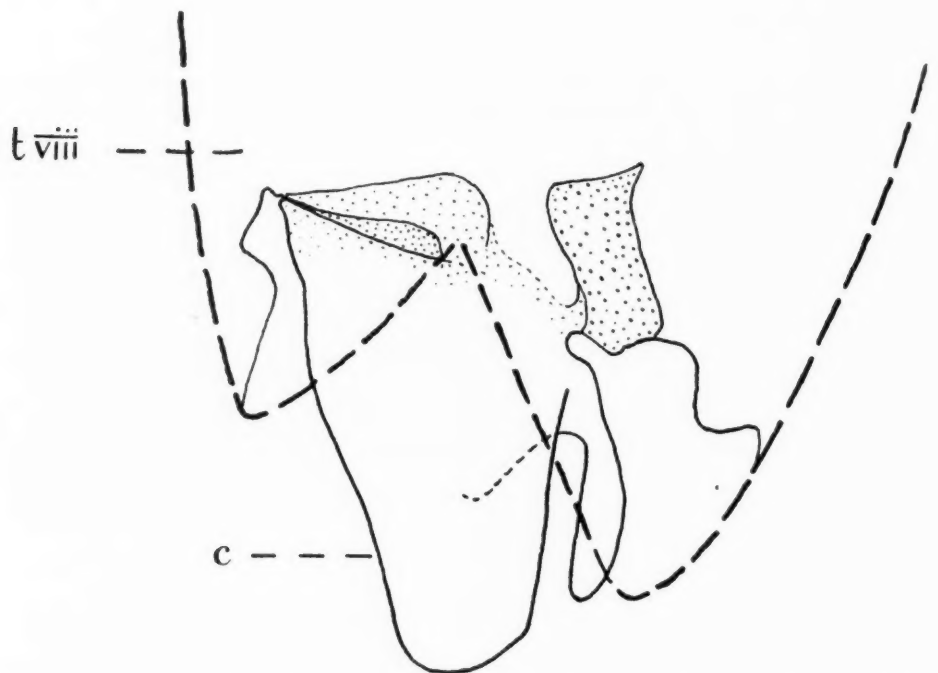


FIG. 22. *Eretmopodites chrysogaster*, Grah., posterior extremity of abdomen of female, lateral view.  $\times$  c. 185.

projecting slightly. Eighth segment not withdrawn within the seventh, sternite projecting slightly further back than the tergite and with its posterior margin deeply notched in the middle. Ninth segment as usual much reduced, chitinised plates rather strong, arranged as shown in the figure. Cerci with blunt or truncated ends; average length about  $200\mu$ , breadth about  $100\mu$ . Ventral process of the tenth segment shorter than the cerci, deeply notched, bearing on each side numerous setae, one pair very strong. Spermathecae three, highly chitinised, sub-spherical; the middle one is the largest and has a diameter of about  $110\mu$  or more, the lateral ones are a little smaller, and are usually, but not always sub-equal, and have a diameter which in the specimens examined ranged from  $91\mu$  to  $114\mu$ , average  $97\mu$ . The commencement of the ducts is chitinised for only a short distance, about  $6\mu$ ; and there are a few pale spots round it at the base of the spermathecae.

*E. quinquevittatus*, Theo. (fig. 23). One specimen. Apparently

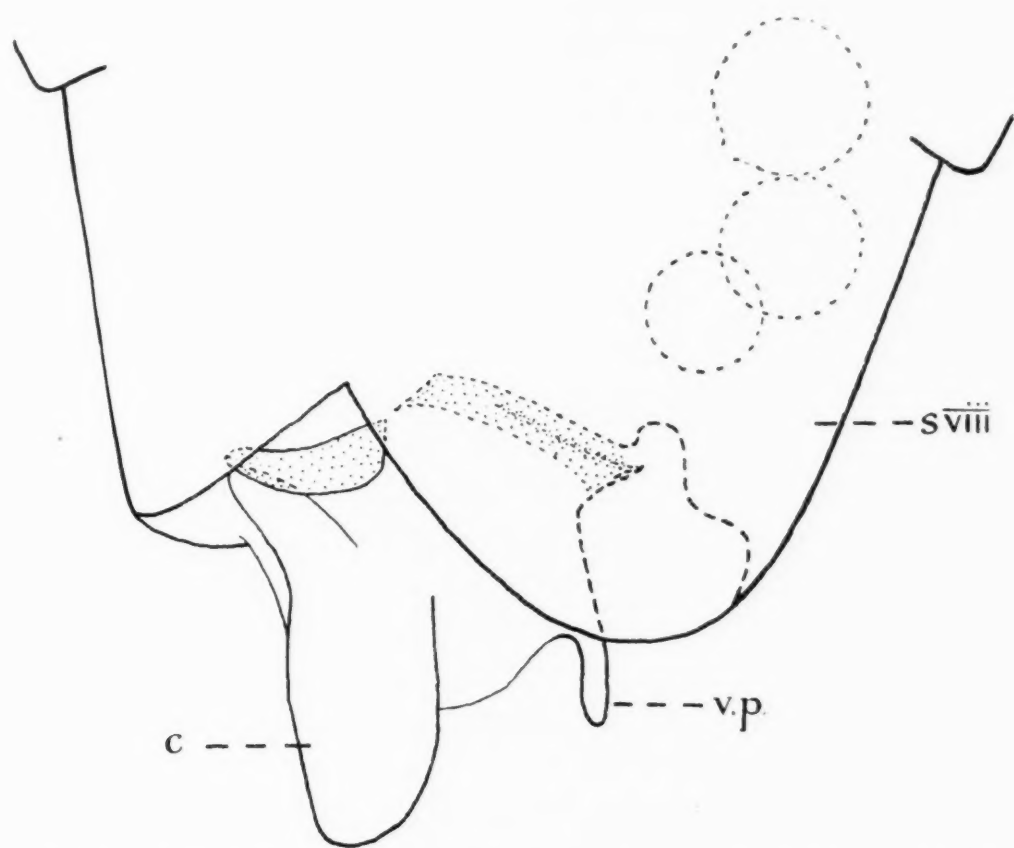


FIG. 23. *Eretmopodites quinquevittatus*, Theo., posterior extremity of abdomen of female, lateral view.  $\times$  c. 185.

almost indistinguishable from *E. chrysogaster*; but in the specimen examined the cerci were slightly smaller, about  $170\mu$  by  $87\mu$ , and so were the spermathecae, the diameters of which were about  $72\mu$ ,  $91\mu$ , and  $68\mu$  respectively, and no part of the ducts appeared to be chitinised.



## NOTES ON AUSTRALIAN CESTODES

BY

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AND

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## V. THREE CESTODES FROM THE BLACK SWAN

The three following species of Cestodes were found in the intestine of *Chenopsis atrata*, Lath. (the Black Swan), several of which were examined at Townsville, North Queensland:—

- (1) *Nematoparataenia paradoxa*, n. g., n. sp.
- (2) *Echinorhynchotaenia nana*, n. sp.
- (3) *Hymenolepis lanceolata* (Bloch, 1782), Weinland, 1858.

- (1) *Nematoparataenia paradoxa*, n. g., n. sp.

On a single occasion about twenty specimens of this worm were obtained.

## EXTERNAL ANATOMY.

The worm measures about 9 mm. in length and 4 mm. in breadth except at the posterior extremity, where it expands into an oval saccular portion measuring about 0.8 mm. in length by 0.6 mm. in breadth.

The cuticle exhibits no trace of segmentation, even under high magnification. In cross-section the worm is circular with a ventral indentation (figs. 3 and 4).

*Head.* The head is armed with four suckers measuring  $80\mu$  to  $100\mu$  in diameter. They are borne on short pedicles about  $100\mu$  long, which are situated about  $200\mu$  from the anterior extremity. The anterior end of the head is occupied by a wide cup-shaped cavity about  $400\mu$  deep, bearing round its margin twelve flattened tentacular processes with minute spines about  $2\mu$  long closely arranged around their borders. These tentacles are similar to those seen in the various species of the genus *Parataenia*, Linton; they

measure about  $120\mu$  long and  $40\mu$  broad. There is a well marked neck about  $500\mu$  in length, which narrows to about  $300\mu$  in

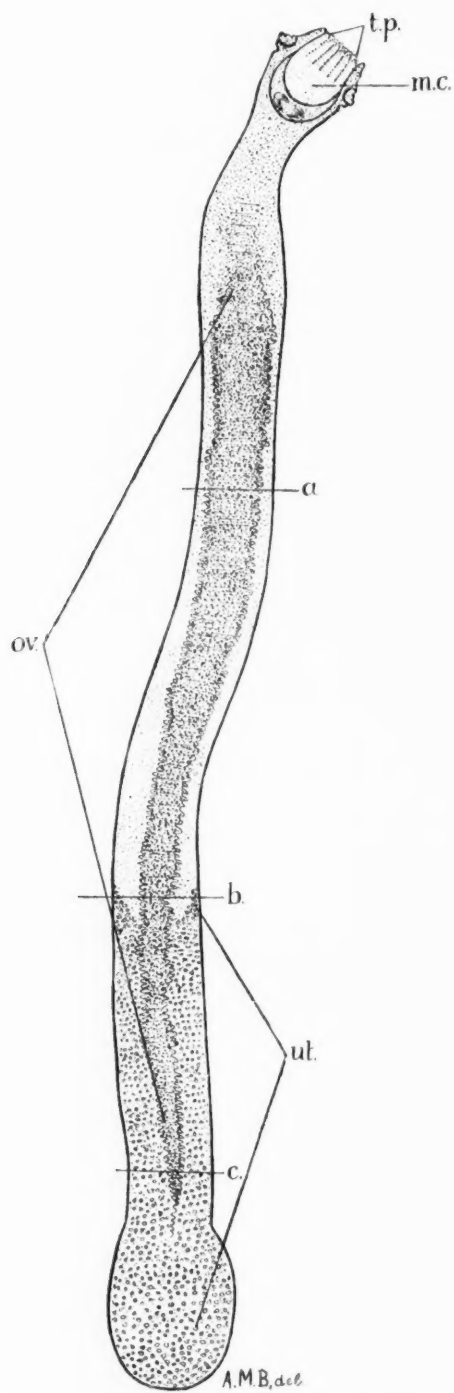


FIG. 1. *Nematoparataenia paradoxa*, n.g., n.sp. Complete worm. *a*—position of fig. 2; *b*—position of fig. 3; *c*—position of fig. 4; *m.c.*—mouth cavity; *ov*—ovary; *t.p.*—tentacular processes; *ut*—uterus.  $\times 17$ .

diameter. The remainder of the worm is cylindrical with a longitudinal groove running along its ventral surface (fig. 1).

## INTERNAL ANATOMY.

*Muscular system.* This consists of a series of separate longitudinal fibres arranged in an irregular double row immediately beneath the cuticle. No transverse or dorso-ventral fibres were seen (figs. 2 and 3).

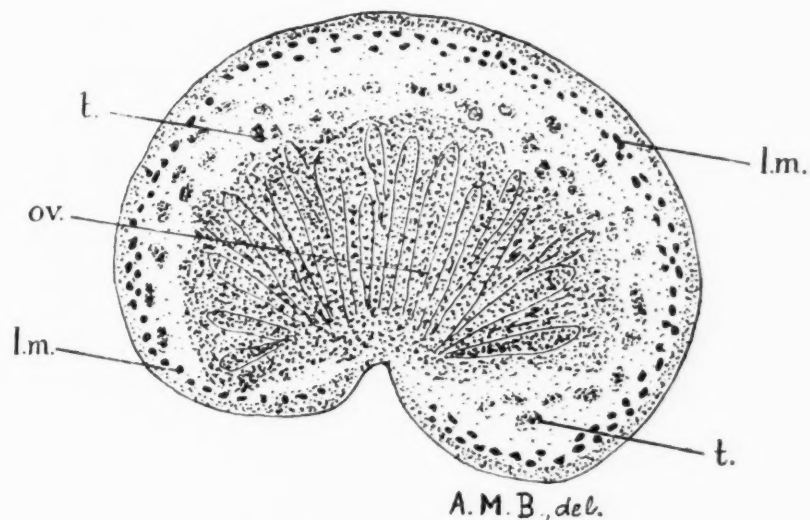


FIG. 2. *Nematoparataenia paradoxa* n.g., n.sp. Transverse section at *a*—fig. 1; *l.m.*—longitudinal muscle fibres; *ov*—ovary; *t*—testes.  $\times 140$ .

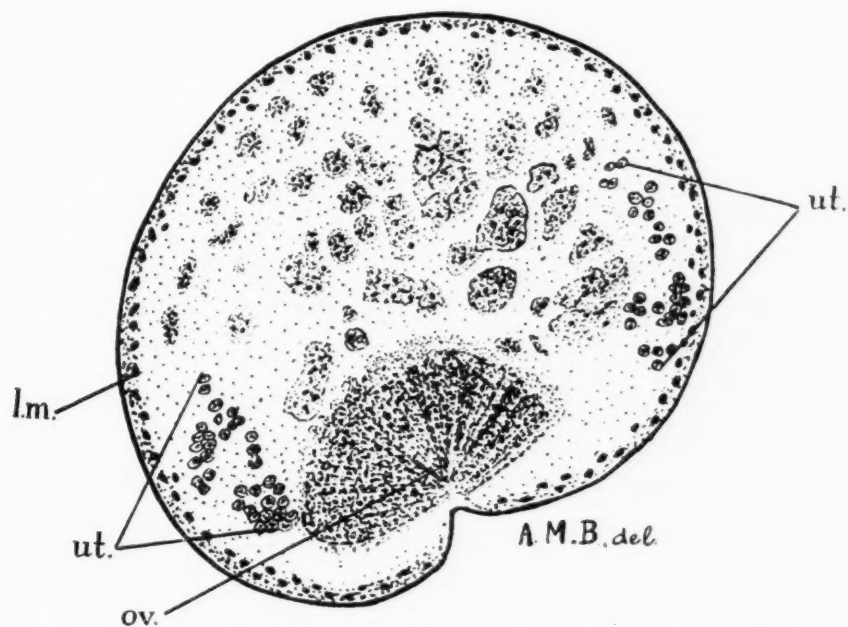


FIG. 3. *Nematoparataenia paradoxa* n.g., n.sp. Transverse section at *b*—fig. 1; *l.m.*—longitudinal muscle; *ov*—ovary; *ut*—uterus.  $\times 140$ .

*Nervous and excretory systems.* No details of these could be made out.

### Genitalia.

*Testes.* The testes are small and extremely numerous, they lie in the dorsal and lateral fields (fig. 2); towards the middle of the worm they begin to become fewer in number.

*Vas deferens.* No vas deferens, cirrus, or genital pore was seen.

*Ovary.* The ovary is situated ventrally and occupies the middle threequarters of the worm's length. In whole mounts the ovary shows no trace of segmentation, except that the lateral margins are serrated (fig. 1); in cross-section it is fan-shaped, the lobes radiating dorsally and laterally from a central point opposite the ventral groove; towards the posterior it gradually atrophies.

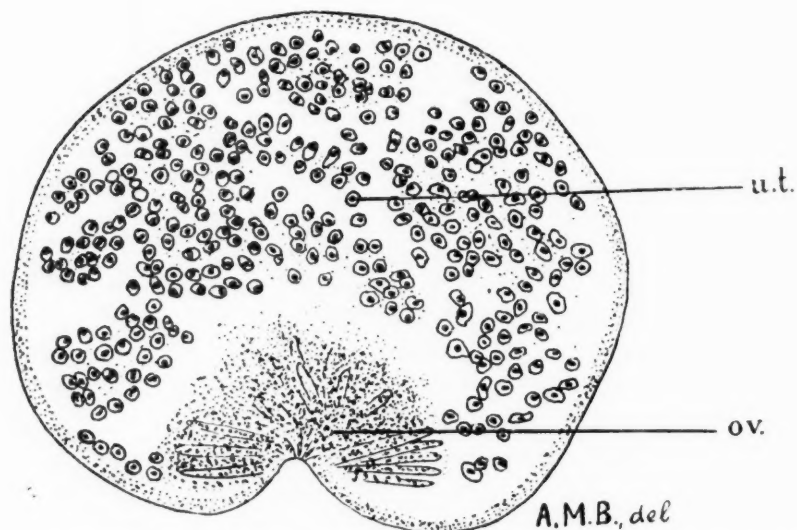


FIG. 4. *Nematoparataenia paradoxa* n.g., n.sp. Transverse section at *c*—fig. 1; *ov*—ovary; *ut*—uterus.  $\times 140$ .

*Vagina and receptaculum.* These structures were not seen.

*Uterus.* The uterus begins about the junction of the middle and posterior thirds of the worm. It first appears at each side close under the cuticle, and as the ovary atrophies the two lateral limbs of the uterus gradually increase in size until they unite, and finally it occupies the whole of the body.

*Eggs.* The eggs are circular and measure about  $10\mu$  in diameter; further details could not be determined.

### DIAGNOSIS.

This worm resembles *Parataenia medusia*, Linton (1890), only in its possession of tentacular processes on the head. It also bears a superficial resemblance to *Nematotaenia dispar*, Lühe (1899) in being unsegmented. Apart from these slight resemblances to the

above two species, this worm has characters entirely different from any known worm; this necessitates its being placed in a new genus, which we have named *Nematoparataenia*, and of which the following is the definition:—

*Nematoparataenia*, n.g.

Cylindrical worms with four suckers, and a number of digitate processes on the head. No trace of internal or external segmentation.\* Type species *Nematoparataenia paradoxa*.

The type species is in the Museum of the Liverpool School of Tropical Medicine.

(2) *Echinorhynchotaenia nana*, n. sp.

About twenty specimens of this worm were obtained. Unfortunately the material was in very poor condition, so a full description is not possible.

EXTERNAL ANATOMY.

The largest worm measured about 2 cm. in length and including the cuticular expansions, which occur on the posterior borders of the segments, 1.7 mm. in breadth; the breadth of the worm without these expansions is about 1.3 mm.

*Head.* The head is about 1.5 mm. broad and 2.3 mm. long. Viewed anteriorly it is square, with rounded corners; each corner is occupied by a very strongly developed sucker looking almost directly forwards, and with a diameter of about 450 $\mu$ . In the centre of the anterior surface there is a small pit. When viewed from the side, the anterior surface is bluntly rounded, and the central pit, which is almost 300 $\mu$  deep, is seen to lie anterior to the suckers. Behind each sucker is a lappet, as in *Anoplocephala perfoliata* (Goeze, 1782), Blanchard, 1848. Behind the lappets the head narrows gradually to a width of about 600 $\mu$ , at which point it is sharply marked off from the narrower anterior segments, which it tends to overlap, by a cuticular collar-like ring. There is no neck (fig. 5).

\* Although we were unable to see definite signs of segmentation, it should be noted that all our specimens were fully gravid and, therefore, old, and it is quite possible that in younger worms there would be segmentation in the internal organs.



*Segments.* The segments are broader than long, the most fully developed being 2 mm. broad and  $200\mu$  long. They are like a number of saucers placed one within the other with the concavity facing posteriorly. This appearance is caused by the whole circumference of the posterior borders of the segments being provided with cuticular expansions about three times as long as the segments themselves. In cross-section the segments of the anterior two-thirds of the worm are nearly circular, whilst those of the posterior third are oval. The genital pores are unilateral and open on the right side.

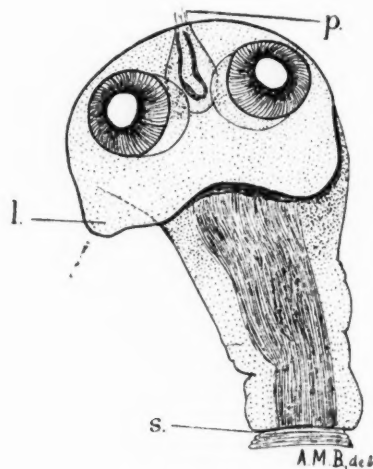


FIG. 5. *Echinorhynchotaenia nana*, n.sp. Scolex. *l*—lappet; *p*—fragment of proboscis; *s*—beginning of strobila.  $\times 17$ .

#### INTERNAL ANATOMY.

*Muscular system.* The longitudinal muscle is disposed in two layers, an outer feebly developed layer consisting of a few small bundles, and a relatively enormously developed inner layer measuring  $300\mu$  in thickness (fig. 6). External to these are a few transverse fibres. No dorso-ventral fibres were seen. Four strands from the internal longitudinal layer run one to each sucker; the latter organs are extremely muscular, and in some specimens had actually fallen out of the scolex and appeared as almost spherical solid bodies.

*Nervous system.* There is a single lateral nerve on each side of the body lying external to the excretory vessels.

*Excretory system.* A number of excretory tubes can be seen in the head, and these unite to form two lateral vessels on each side. The two lateral vessels are of about the same diameter, and one lies directly dorsal of the other.

### Genitalia.

*Testes.* The testes are three in number and they lie behind the ovary in the same transverse plane, two being on the aporal side. In full development they measure about  $60\mu$  in diameter.

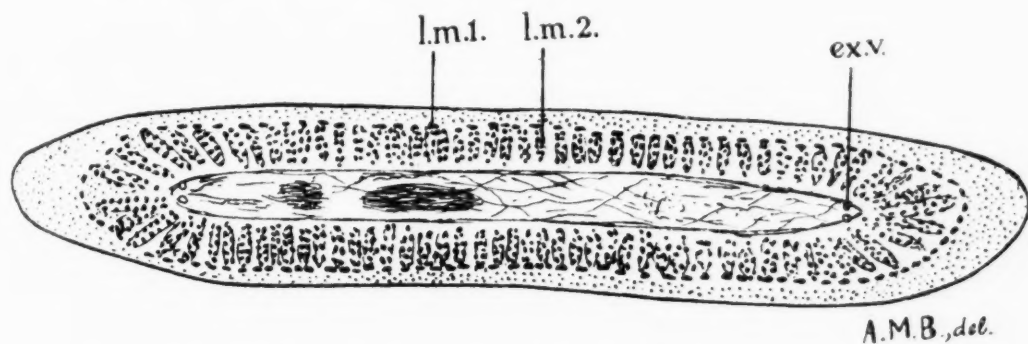


FIG. 6. *Echinorhynchotaenia nana*, n.sp. Transverse section towards posterior part of worm. *ex.v.*—excretory vessels; *l.m.1.*—outer longitudinal muscle layer; *l.m.2.*—inner longitudinal muscle layer.  $\times 70$ .

*Vas deferens.* The vas deferens expands into a fairly large vesicula seminalis lying anterior to the mesial end of the cirrus pouch and connecting with the latter organ by a narrow duct. The cirrus pouch is  $500\mu$  long and  $80\mu$  broad, extending almost half-way across the segment. The cirrus is as long as its pouch and ends in a club-shaped extremity, the extreme end of which is surrounded by a small sphincter muscle. The external surface of the cirrus is closely

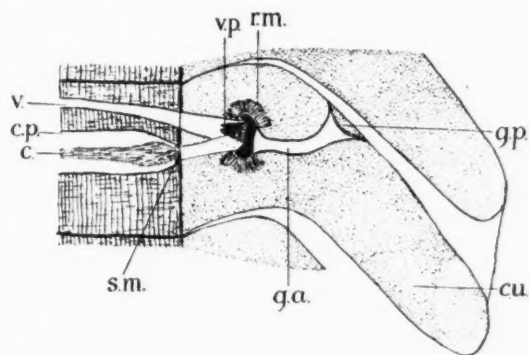


FIG. 7. *Echinorhynchotaenia nana*, n.sp. Termination of sex ducts. *c.*—cirrus; *c.p.*—cirrus pouch; *cu.*—cuticle; *g.a.*—genital atrium; *g.p.*—genital pore; *r.m.*—retractor muscle; *s.m.*—sphincter muscle at tip of cirrus; *v.*—vagina; *v.p.*—vaginal plug.  $\times 40$ .

covered with minute spines. From the lateral border of the segment the male duct extends into the cuticle as a thin-walled tube, and it ends at its junction with the vagina which occurs about the centre of the cuticular expansion. From this junction the genital atrium runs laterally to open on the anterior surface of the cuticular prolongation about the junction of its inner and middle thirds (fig. 7).

*Ovary.* The material was in such a bad state of preservation that details relating to the ovary and vitelline glands could not be made out. The ovary is centrally situated in front of the testes, and all that could be seen was a number of acini, each measuring about  $30\mu$  in diameter.

*Vagina and receptaculum.* The vagina opens into the genital atrium immediately ventral to the male pore, and lying in its open end is a solid conical plug with a broad base (fig. 3). This plug is inserted into the slightly funnel-shaped opening of the vagina, and around the opening is a strongly developed muscle, which from the radial arrangement of its fibres probably acts as a retractor, drawing the walls of the vagina away from the plug. From the pore the vagina passes inwards anterior to the cirrus pouch, narrowing slowly until just internal to the excretory vessels it expands into a club-shaped receptaculum seminis, which runs as far as the median plane.

*Uterus.* The uterus is a simple transverse sac loosely packed with eggs.

*Eggs.* The eggs are circular and measure  $40\mu$  in diameter, and the oncosphere measures  $32\mu$ .

#### DIAGNOSIS.

Führmann (1909) erected the genus *Echinorhynchotacnia* to accommodate a species which possessed a proboscis-like rostellum armed with spines. Our worm closely resembles Führmann's species in its general anatomy except that the characteristic proboscis had been apparently torn out in all our specimens, but the appearance of the head, with a few ragged fibres protruding from the central pit, leaves no room for doubt that a proboscis has been present. The points in which our species differs from Führmann's *E. tritesticulata* are the following:—

	<i>E. tritesticulata</i>	<i>E. nana</i> , n.sp.
Length ... ..	30 cm.	2 cm.
Breadth ... ..	4-5 mm.	1.7 mm.
Lappets ... ..	absent	present
Apparatus at vaginal pore ... ..	absent	present
Genital atrium ... ..	absent	present
Position of genital pore ... ..	On anterior of lateral border of segment.	On anterior surface of cuticular expansion

We, therefore, consider ours a new species, and name it *Echinorhynchotaenia nana*.

The type specimen is in the Museum of the Liverpool School of Tropical Medicine.

(3) *Hymenolepis lanceolata* (Bloch, 1782), Weinland, 1858.

This cestode was found on four occasions. Many hundreds of specimens were obtained, and as they showed a wide variation in size and development, it is proposed to discuss these variations, since apparently they have not been noted in previous descriptions of the species.

The largest specimen was 55 mm. in length with a maximum breadth of 5 mm., and from these dimensions there were worms of every gradation in size down to specimens only 11 mm. in length by 0.3 mm. in breadth; that this difference in size is not altogether due to different ages of the specimens is shown by the fact that many of the smallest worms had a fully gravid uterus in their posterior segments.

Some of the smaller worms have only a part of the genitalia present. That is, either the male or female organs may be completely absent, but in no case was a worm seen in which both sets of glands were absent. In some without testes the uterus contains eggs; probably this is brought about by cross-copulation between different individuals. It may be held that the testes were originally present and have atrophied, but this is unlikely, as in the larger normal worms testes and ovaries are present together in all of the mature segments. In these small varieties the muscular system is poorly developed, with the result that the worms are very thin and diaphanous when compared with the larger ones. Another abnormality which was frequently observed was that the segments immediately behind the scolex rapidly increased in breadth in the normal manner, but after about the twentieth segment, instead of continuing to increase they became successively narrower for about a similar number of segments, after which the usual gradual and continuous increase took place.

At first glance it would appear that worms of different species were included under the one head, but that this is not the case is shown by the following points:—

(1) The scolex and the few segments immediately following it are the same in all cases.

(2) The cirrus, when present, is always of the same relative length and shape, no matter what the size of the worm.

(3) The male and female genitalia occupy the same relative positions in the segments, whether present complete or only in part.

(4) When a long series of material is examined, a regular sequence from the largest to the smallest worms can be obtained.

As normally developed worms departed in no particular from previous descriptions of the species *H. lanceolata*, detailed anatomy has not been given.

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# THE INCIDENCE OF A DISEASE IN POPULATION GROUPS, THE NUMBER OF PEOPLE IN WHICH IS KNOWN OR UNKNOWN

BY

J. W. W. STEPHENS

*(Received for publication 25 May, 1922)*

As an example of the 'incidence,' 'occurrence,' or 'distribution' of cases of a disease in one or more groups, such as age-groups of a population, the number of people in which is *unknown*, we may take the following. Of a total of twenty cases of influenza, let us suppose that ten occurred in Group A and ten in Group B, then the respective incidences ten and ten are equal, and the number that occur in each group per one hundred cases, viz., fifty and fifty, are also equal.

As an example of the 'incidence,' 'occurrence' or 'distribution' in age-groups, the number of people in which is *known*, we may take the following. Of a total of twenty cases of influenza, let us suppose that ten occurred in Group A, containing one hundred people, and ten in Group B, containing fifty people, then the incidences are 10 per cent. and 20 per cent. respectively (and the ratios of the incidences per one hundred cases 33 per cent. and 66 per cent. respectively).

It will be evident that the term 'incidence' has been used here in two different senses. In the first sense of the term, 'incidence,' it is only the number of cases that is known. In the second sense, when not only the number of cases but also the number of people among whom the cases occur is known, the term is applied to a figure expressing the number of cases that occur per one hundred people in each group.

To emphasise the distinction in meaning between these two uses of the term 'incidence,' it would seem advisable to confine the term 'incidence' to the use of the term in the first sense, and the term 'incidence rate' to the use of the term in the second sense.

In practice, however, certain deductions are often made when the number of cases alone is known, which can, as we shall see, be only justifiably made when the number of people in the groups is also known, *i.e.*, when the 'incidence rate' can be calculated.

In regard to 'incidence,' the larger the group, the larger (*ceteris paribus*) is the incidence. In regard to 'incidence rates,' the factor of unequal size of the groups, if it exists, is eliminated, as the rate is calculated for one hundred people in each group.

The above examples may be tabulated as follows, using the words incidence and incidence rates in the sense defined above.

TABLE I.

Shewing distinction between incidence and incidence rate.

		1	2	3	4	5	6	7
Group		Total number of people that occur in each group (Census)	Number of people that occur in each group per 100 people (Census)	Incidence, <i>i.e.</i> , total number of cases observed that occur in each group	Number of cases that occur in each group per 100 cases	Incidence rate, <i>i.e.</i> , number of cases occurring among 100 people in each group	Ratios of the incidence rates to one another	Ratios of the incidence rates to one another per cent.
Ex. 1.	A	...	...	10	50	...	...	...
	B	...	...	10	50	...	...	...
Ex. 2.	A	100	66.6	10	50	10	1	33.3
	B	50	33.3	10	50	20	2	66.6

From the second example in the table we see that the 'liability to attack' of a person in Group A is 10 per cent. and in Group B 20 per cent., *i.e.*, it is twice as great in Group B as in Group A. This fact cannot, however, be deduced from the figures in the first example, because, although the number of cases is the same as in the second example, nothing is known as to the number of people among whom the cases occurred. It is the incidence rates (actual or relative) that are of importance if we are studying what may be termed the 'real incidence' of the disease on a group.

## DIABETES

We find recorded in Osler and Macrea, *System of Medicine*, second edition, p. 675, the age-group incidence of three hundred and thirty-five cases of diabetes in Baltimore (column 3) from which can be readily calculated the age-group incidences per one hundred cases (column 4). The figures for the age-group, distribution or incidence of the population of Baltimore per one hundred people are not given, so that for purposes of illustration I have used those of Liverpool as deduced from the 1911 census (Table II, column 2).

TABLE II.

Showing incidences and ratios of incidence rates in Diabetes.

		1	2	3	4	5	6	7
Age-Group		Total number of people that occur in each group (Census)	Number of people that occur in each group per 100 people (Census)	Incidence, i.e., total number of cases observed that occur in each group	Number of cases that occur in each group per 100 cases	Incidence rate, i.e., number of cases occurring among 100 people in each group	Ratios of the incidence rates to one another	Ratios of the incidence rates to one another per cent.
1-10	...	...	23.3	8	2.18	...	0.0935	0.86
11-20	...	...	18.9	25	7.34	...	0.3883	3.59
21-30	...	...	16.7	44	13.1	...	0.7245	6.71
31-40	...	...	15.8	61	18.2	...	1.1519	10.67
41-50	...	...	11.2	69	20.6	...	1.8392	17.04
51-60	...	...	7.3	89	26.5	...	3.6301	33.64
61-70	...	...	4.5	33	9.8	...	2.1111	19.47
71-80	...	...	2.0	6	1.7	...	0.8500	7.87
81-	...	...	0.3	0	0.0	...	0.0	0.0
		...	100.0	335	99.42	...	10.7886	99.85

If we were dealing with the total number of people in each group (column 1) instead of the number per one hundred of the population, and divided a figure in column 3 by the corresponding figure in column 1 and multiplied the result by one hundred, the figures obtained would represent the incidence rates, i.e., the incidence per

one hundred people (column 5). But, in the present case, where we have divided the percentage figures in column 4 by the percentage figures in column 2, the resulting figures (column 6) represent simply the *ratios*\* which the incidence rates bear to one another, and from these we can easily calculate the ratios, when the sum of the ratios is one hundred (column 7). Thus, to refer to Table II (column 7), we see that of one hundred cases of diabetes about thirty-three would occur among so many people in the age-group 51-60, while about half that number (17.04) would occur among the *same number* of people in the age-group 41-50, whereas, considering the incidence merely (column 4), it is about the same in the two groups, viz., 20.6 and 26.5 respectively.

### INFLUENZA

The following example (Table III) is taken from Nothnagel's *Encyclopædia of Practical Medicine*, English Edition. Article 'Influenza,' p. 571. The actual figures for the case incidence and the population incidence in the various groups are not given, but only the percentage incidences in each case, in the form of graphs. The figures are only approximately correct, as it was not possible to calculate them exactly from the graphs. As in Table II, by dividing the percentages in column 4 by the corresponding ones in column 2, we get a series of figures (column 6) which represent the ratios which the incidence rates bear to one another, and in column 7 the ratios of these rates per cent. Thus, the 'liability to attack' (column 7) in the age period 21-30 is slightly more than twice as great as in the age-period 51-60, but what the actual figures for liability to attack are it is impossible to say, as it is only the percentage and not the actual number of people in the groups that is known. The figures in column 7 are not comparable with those in column 4; strictly speaking, no conclusions as to 'liability to attack' can be based on the figures in column 4 by themselves. It is only if we assume *some* knowledge of the number of people in the groups that the case incidence figures have any value in this respect.

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\*The *ratios*, but of course not the same actual figures, in this column could equally well be got by dividing the figures in column 3 by those in column 2.

Thus, we could probably infer that the liability to attack was greater in the 21-30 period than in the 11-20 period, because we *assume* that the population of the 21-30 period is probably not twice that of the

TABLE III.

Showing incidences and ratios of incidence rates in Influenza.

		1	2	3	4	5	6	7
Age-Group		Total number of people that occur in each group (Census)	Number of people that occur in each group per 100 people (Census)	Incidence, i.e., total number of cases observed that occur in each group	Number of cases that occur in each group per 100 cases	Incidence rate, i.e., number of cases occurring among 100 people in each group	Ratios of the incidence rates to one another	Ratios of the incidence rates to one another per cent.
1-10	...	...	19	...	8	...	0.421	5.86
11-20	...	...	16	...	15	...	0.937	13.05
21-30	...	...	21	...	32	...	1.523	21.07
31-40	...	...	16	...	20	...	1.250	17.40
41-50	...	...	12	...	14	...	1.166	16.23
51-60	...	...	8	...	6	...	0.750	10.44
61-70	...	...	5	...	4	...	0.800	11.14
71-80	...	...	3	...	1	...	0.333	4.63
		...	100	...	100	...	7.180	99.82

11-20 period; but we can only make accurate deductions, giving relative or actual figures, when we base them on the number of people, relative or actual, in the groups.

### BLACKWATER FEVER

It has been commonly stated that the liability to an attack of blackwater fever is greater in persons infected with malignant tertian parasites than in those infected with simple tertian or quartan parasites. These statements are based on the particular parasites present in so many *cases* of blackwater fever, but, as we have shown



above, no conclusions can be drawn as to liability to attack unless we have population data as well.

The case before us is parallel with the two examples we have already considered, though here, instead of age-groups, we have groups of persons (malaria cases) infected with the malignant tertian and simple tertian parasites respectively (Table IV). The data are

TABLE IV.

Showing relative liability to an attack of Blackwater fever of persons infected with malignant tertian and simple tertian parasites respectively.

		1	2	3	4	5	6	7
	Parasite Group	Total number of case of Malaria that occur in each group	Number of cases of Malaria that occur in each group per 100 cases of Malaria	Total number of Blackwater cases observed that occur in each group	Number of cases of Blackwater that occur in each group per 100 cases of Blackwater	Incidence rate, i.e., number of cases of Blackwater that occur among 100 cases of Malaria in each group	Ratios of incidence rates to one another	Ratios of incidence rates per cent.
Ex. 1	Malignant tertian ...	...	74	...	76.4	...	1.032	53.2
	Simple tertian ...	...	26	...	23.6	...	0.908	46.8
		...	100	...	100	...	1.94	100.0
Ex. 2	Malignant tertian ...	...	68.45	...	54.03	...	0.789	35.1
	Simple tertian ...	...	31.56	...	45.96	...	1.456	64.9
		...	100.00	...	99.99	...	2.245	99.9

taken from a paper in the *Annals of Tropical Medicine and Parasitology*, Vol. VII, December, 1913, p. 487, in which I have summarised the data of Deeks and James, and Lovelace, respectively.

As before, the figures in column 6 are got by dividing those in column 4 by the corresponding ones in column 2. The figures in column 7 are then calculated for one hundred cases.

In the first example, the incidence rate of blackwater fever in malignant tertian infections is only slightly greater than that in simple tertian infections.

In the second example, the incidence rate in simple tertian infections is nearly twice as great as that in malignant tertian infections.

We are not concerned here with the discrepancy between the results, but with the fact that in each case deductions based solely on the incidence, *i.e.*, occurrence of malignant tertian or simple tertian parasites in the blackwater *cases*, would have led to different but erroneous conclusions.

A reference to the current text-books of Tropical Medicine would afford many other examples of a similar kind, where conclusions are drawn from a knowledge of the number of cases only, in the absence of any knowledge of the number of people among whom the cases occur.



# THE EXPERIMENTAL INFESTATION OF *PHYSOPSIS AFRICANA*

BY

F. G. CAWSTON, M.D., Cantab.

*First Streatfeild Research Scholar*

(Received for publication 25 May, 1922)

Some notes on the experimental infestation of *Physopsis africana* in Natal may prove of use for reference to workers in other parts of the world who are engaged in the study of the life-history of the schistosomes.

It was some time before I succeeded in keeping this common fresh-water snail alive for any length of time under artificial conditions. The glass jars in which I observed the growth of young examples proved unsuitable for more than a few days; but wooden tubs, kept out of doors in a shady place, answered the purpose very well, and I was able to secure all I needed whilst the experiments were in progress. I did not find it necessary to change the water in the tubs, which contained a few water-plants and an increasing amount of decomposing leaves and small pieces of wood which fell in occasionally. The snails had, therefore, plenty of shade, whilst the water never became too hot, as it tends to do in a glass jar if placed in the sun. As 'millions' had been observed feeding on young snails, and as one did not wish to interfere in any way with the free ventilation of the water, nothing was done to prevent the breeding of mosquitoes. The surface area of the water was approximately double that of the bottom of the tubs.

It is possible that the rate of growth was handicapped by the food supply—and I have not succeeded in getting the common variety of water-lily to thrive in wooden tubs—but, even under what appear to be very favourable conditions, I do not find it possible to obtain examples large enough for experimental purposes under five months in Natal, and I should gather that by far the majority of

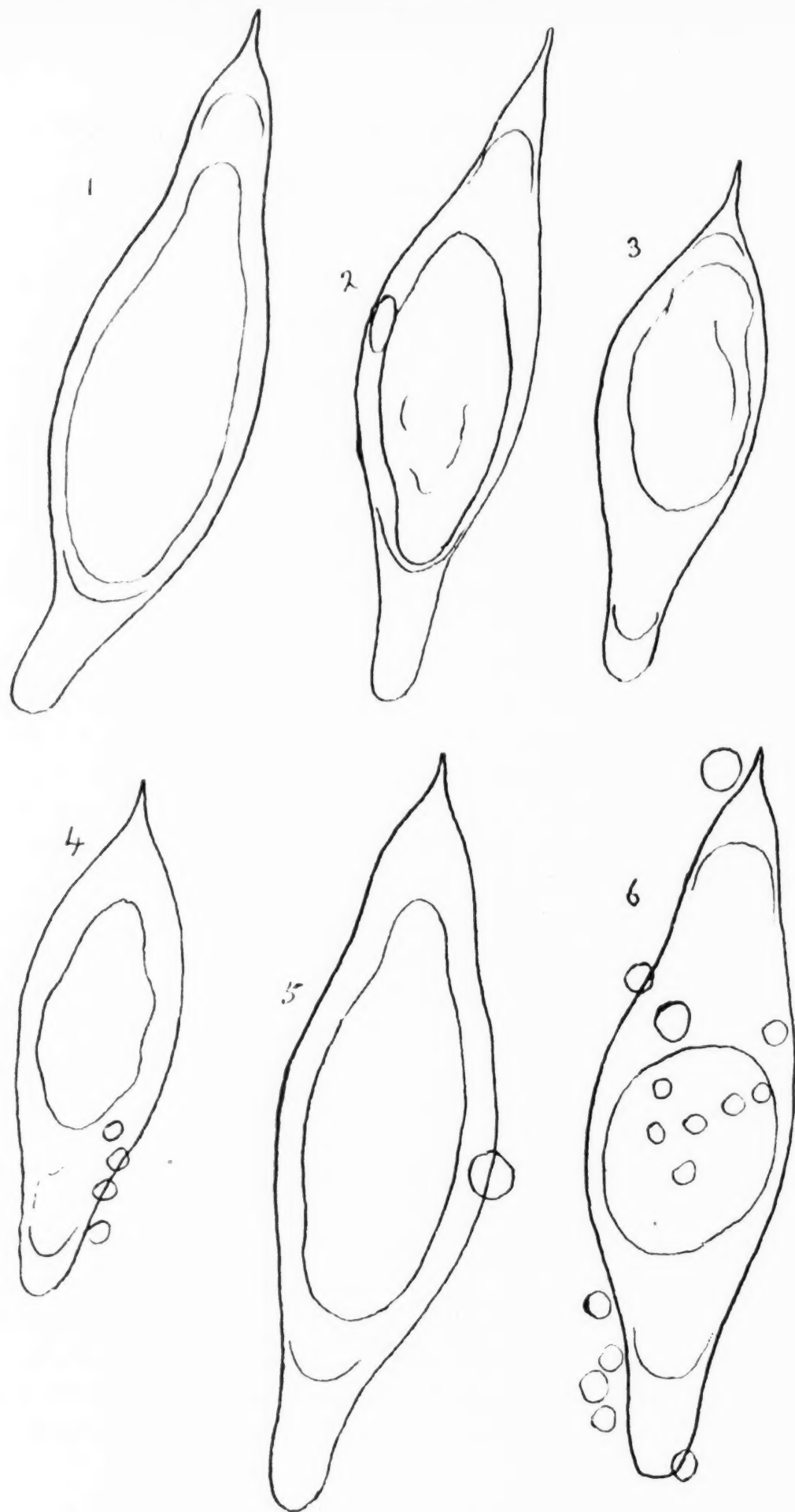


FIG. 1. Ova from urine of Natal boy. Note solid, long, rounded extremities. Average length 0.22 mm.; exceptional length 0.2625 mm.; abnormally bent end in 1. Living miracidia in 1, 2 and 5. Degenerative effects of emetine in 3, 4 and 6.



infested examples that I have found in the rivers and pools of South Africa were at least a year old. I have noted the presence of apparently mature cercariae in very small specimens from Natal rivers, and in some which were experimentally infested forty-six days before and the shells of which measured only 6.5 and 7.0 mm. in length; but it is rare to find such small specimens infested.

When required for the experiments, a number of well-developed examples, about 12 mm. in length, were selected and placed in a glass jar containing fresh water. The urine of a Bilharzia patient was then secured, and the ova collected by centrifugalising the specimen. The ova were examined microscopically, identified by

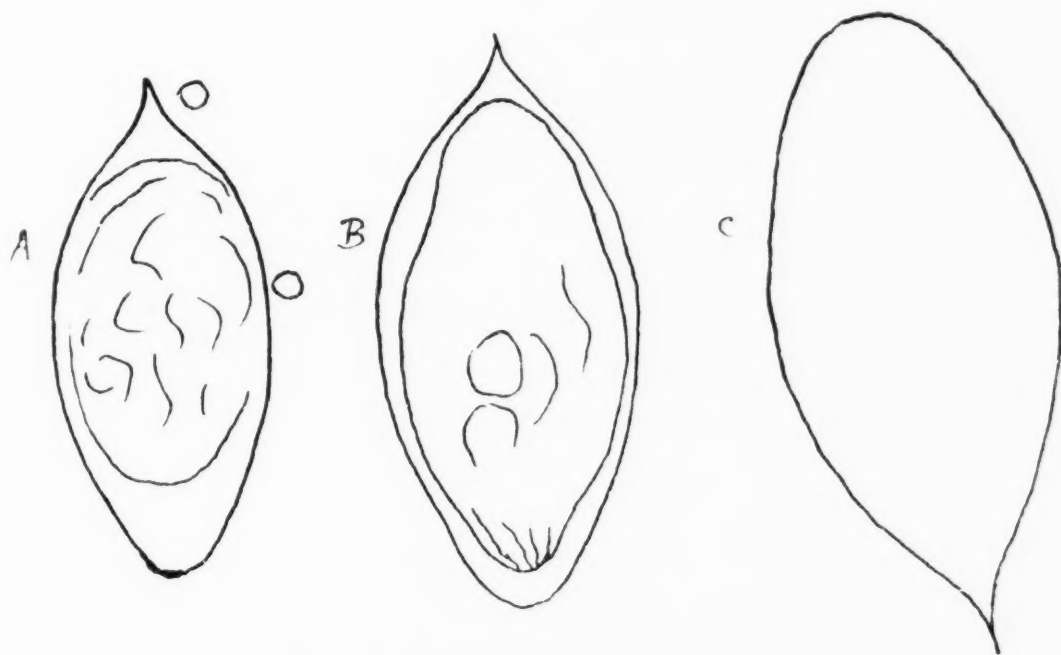


FIG 2. Ova of *S. haematobium* from same urine, showing (B) living miracidium about to hatch, and (A) miracidium degenerated under the influence of emetine.

means of their shape, size and spine as those of *Schistosoma haematobium*, and, as soon as the contained miracidia were seen to be ready to hatch, were emptied into the jar containing the snails and placed in a good light for a few hours. At the end of twenty-four hours the snails were then generally placed in a small wooden tub.

Some of the snails which had been thus exposed to infestation were placed in some dark glass jars containing a few decomposing leaves. The water in these jars were continually replaced by drops

from a glass tubing connected with a large tub containing water-weeds. Whilst the snails were thus continually receiving fresh water laden with food, the water was gradually escaping through a regulated syphon tube. This arrangement answered well for a few snails at a time.

Long spindle-shaped ova resembling those of *Schistosomum bovis* were found in the urine of two Natal-born Indian boys, associated with the typical ova of *Schistosoma haematobium*. Both varieties were added to water containing *Physopsis* which had been kept free from all other possible chances of infection by miracidia.

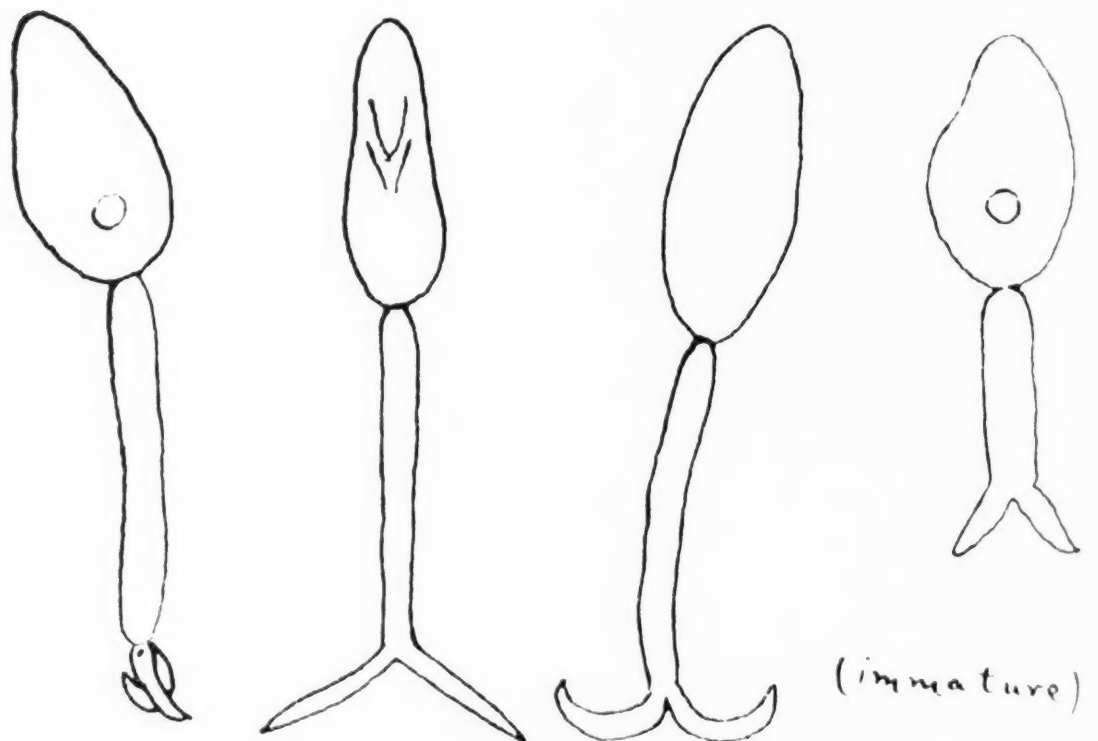


FIG. 3. Cercariae present in *Physopsis africana* 63 days after being exposed to the above ova. ( $\times 2$ .)

To imitate natural conditions, as far as possible, it is necessary to wait until cercariae are escaping into the surrounding water before using them for the experimental infection of animals. Although I have found apparently mature cercariae in *Physopsis* which has been exposed to miracidia only a fortnight before, I have never found the cercaria outside a snail until the development has been allowed to progress for thirty-five days, and it is probably better to keep the infested snails living for several months before dissecting them.

To ascertain whether the experimental infestation has been

successful in specimens one does not wish to destroy, it is best, as Dr. J. G. Becker once pointed out to me, to place individual snails in clean test-tubes in a good light, or even break off a minute portion of the shell over the liver. There may be certain conditions in the surrounding medium that encourage the mature cercariae to work their way out of the infested snail; but I have carefully examined specimens for several days, up to the sixty-fourth day, without any sign of free-swimming cercariae, when dissection revealed the presence of a number of mature cercariae within the liver substance.

Among about thirty individuals that I have found infested with schistosomes within one or two months after being exposed to the ova of *Schistosoma haematobium* and those resembling *S. bovis*, I have never seen any cercaria which shows eye-spots, development in rediae or possessing the long prongs that some of the schistosomes that I have found in *Physopsis* in the Natal rivers occasionally do. In every instance, when mature, the experimentally produced cercaria in *Physopsis* exposed to infection from the urine of a Bilharzia patient was 0.525 mm. in total length, possessed prongs which were about a quarter the length of the tail, and in other respects resembled the cercaria of *S. haematobium*.

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# NOTES ON CULICIDAE IN VENEZUELA, WITH DESCRIPTIONS OF NEW SPECIES

## PART II

BY

ALWEN M. EVANS

(Received for publication 31 May, 1922)

## PLATE XI

Since the completion of a previous paper (1921) we have received, through the kindness of Dr. Chacin and Dr. M. Núñez Tovar, a number of consignments of mosquitoes collected, during the Autumn of 1921, from the regions surrounding Caracas and Maracay.

Most of the species represented are common, and of wide distribution, but among them are a new species of the *Arribalzagia* group of *Anopheles* and a very distinctive new species of the *Janthinosoma* group of *Psorophora*.

*Anopheles albimanus*, Wied.

La Cabrero, Estado Carabobo, ♀ 1; Tapatapa, near Maracay, ♂ 1,  
♀♀ 3; La Barraca, Maracay, ♀♀ 3. Dr. M. Núñez Tovar.

*A. albimanus* var. *tarsimaculata*, Goeldi.

San Francisco, near Maracay, ♀♀ 3; La Cabrero, Estado Carabobo,  
♀♀ 4; La Barraca, near Maracay, ♀ 1; near Maracay, ♂♂ 2, ♀ 1.  
Dr. M. N. Tovar.

A number of specimens occurred in which the condition of the palpi was intermediate between the type *A. albimanus* and the variety *tarsimaculata*. Some specimens have the palpi with the penultimate joint with basal white ring; beyond, black scaled with a number of white scales scattered among the black ones about half-way between base and apex of segment. In other cases the penultimate joint with basal white ring and most of scales on outer side of a much paler brown than the rest of the dark palpal scales.





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*A. argyrotarsis*, R.D.

San Francisco, near Maracay, ♀ 1. Dr. M. N. Tovar.

*A. pseudopunctipennis*, Theo.

San Francisco, near Maracay, ♂♂ 3, ♀♀ 2; La Cabrero, Estado Carabobo, ♀ 1; Tapatapa, near Maracay, ♂ 1, ♀♀ 3; La Barraca, near Maracay, ♀♀ 2. Dr. M. N. Tovar.

*A. maculipes*, Theo.

San Francisco, near Maracay, ♀ 1. Dr. M. N. Tovar.

*Anopheles (Arribalzagia) venezuelae*, sp.n. (Plate XI).

## FEMALE.

*Proboscis* slightly more than 3 mm. Curved ventrally in distal half; labellae conical, dark ochraceous distally, shading to brown at base with a few fine dark hairs; vestiture of dark coppery brown scales roughened basally beneath. *Palpi* shorter than proboscis, clothed with black, spatulate scales sub-erect on basal third, yellow scales forming very narrow rings at apex, at base of last segment, and at middle of long segment; also a few scattered pale scales on distal half. Apex with a tuft of pale yellow hairs. Antennae with long segments densely clothed with fine decumbent hairs, setae of whorls sparse, pale. Third segment with a large patch of flat decumbent scales on upper and inner side on distal half, white proximally, ochraceous golden distally. Tori moderate, dark brown with white scales externally. *Clypeus* large, surface minutely punctate, olive brown proximally, ochraceous pollinose distally. Eyes deep black. Occiput with median groove; integument brown, light grey at margins of eyes and medially. Vestiture of dense upright forked scales, ochraceous in front, with a few whitish ones in middle, dark ones behind; posteriorly and laterally scales very dense, black. Space between eyes with pale narrow scales at borders, and many long creamy forwardly-projecting hairs. A few brown setae projecting forwards from posterior margins of eyes.

*Prothoracic lobes* with erect black scales and a few brownish hairs above; whitish scales below. *Mesonotum* greyish pruinose mottled with brown spots; three large black ocellar spots: two lateral and one posterior involving the scutellum. Vestiture of straw-coloured hairs, many of them arising from pigmented spots. Median area bordered by two

narrow longitudinal yellowish depressed bare stripes, and two wider bare depressions extending from the lateral ocellar spots to the posterior border of the mesonotum. At anterior border a group of strongly curled, pale yellow hairs. *Scutellum* greyish pruinose at sides, along posterior border a median and two lateral groups of yellow hairs and a continuous row of long dark setae. *Postnotum* nude, dark tawny ochraceous at sides with broad brown median stripe. *Pleurae* pale greyish pruinose with five large spots unicolorous with ocellar spots on thorax, dark brown basally. A few flat, white scales medially.

*Abdomen* dorsum uniform mouse grey, vestiture of fine yellow hairs. Distal margins of segments two to seven with a few black scales, and lateral projecting tufts of black spatulate scales with a few white ones near them. Eighth segment with pre-apical band of flat ochraceous scales, and border of flat black scales, and pale yellow hairs. Last segment black scaled. Venter greyish pruinose, with long yellowish hairs at sides of segments, and shorter dark hairs medially. Segments two to seven with broad, white semi-decumbent scales thinly scattered over surface; apical third with dense patches of sub-erect black scales. Venter of eighth segment with golden-yellow, appressed scales. Terminal segment black scaled.

*Wings.* First fork cell one and a half times as long as its petiole; second fork cell about as long as its petiole. *Basal cross vein very narrowly separated from anterior cross vein.* Three large black scaled patches bordered with white scales, with membrane beneath deeply infuscated, the largest median involving the costa, sub-costa and first and second veins; the smallest basal, involving the costa, sub-costa and first vein; the distal one involving the costa, first vein, and both branches of the second vein. Pale scales of wings mostly light yellow except white spots bordering large black patches. Costa mostly black scaled with ten small pale spots, four proximal to large median black patch; scales at apex of costa grey. Sub-costa mostly black scaled with small pale spots opposite those on costa, some decumbent pale scales on basal quarter, largely pale scaled beyond median black patch, apex pale. First vein, in addition to the three large black patches, with black decumbent scales on proximal eighth, between proximal and distal black patch, outstanding scales mostly pale, decumbent scales black; between median and distal black patches, vein mostly pale scaled, with one small black spot and a few scattered black scales. Beyond distal dark patch two grey spots;

apex of vein pale. Second vein: stem beyond median black patch mostly pale scaled, upper branch of fork black scaled on proximal half, beyond with pale scales and a few grey ones; apex grey. Lower branch of fork with a large proximal and apical dark patch, and two smaller dark spots separated by a pale patch. Third vein dorsally mostly pale scaled, with four black spots, two near the base, one apical, and one sub-apical; one or two black decumbent scales on central, pale area. *Third vein from below appearing dark scaled.* Fourth vein mostly black scaled, stem with many pale decumbent scales between the black ones. Proximally, rest of stem with seven pale spots, one at base of fork; branches of fork each with three pale spots; on upper branch the middle white spot elongate. Fifth vein mostly pale scaled, stem with three black spots at base, and many decumbent black scales on distal half. Upper branch of fork with four small and one large black spot, apex black; lower branch with two black spots distally, apex pale, both branches with a few black scales scattered among the pale ones. Sixth vein with seven black spots; apex black. Fringe with twelve pale spots, largest at apex of lower branch of fifth vein. Outstanding scales varying greatly in size and shape; on distal portions of veins lanceolate to narrowly ovate; on basal portions ovate with many very broadly ovate ones on fourth vein and sub-costa, some of those on sub-costa obliquely truncate. (Plate XI, fig. 1).

*Halteres* above densely white scaled with sub-circular dark median area; beneath densely black scaled, stem nude, ochraceous.

*Legs.* Very long and slender. Vestiture black with many white spots and bands. Femora and tibiae densely mottled with white spots. Hind tarsi with ten white rings or spots on first joint, seven on second, four on third; fourth and fifth white ringed at base and apex and in middle (Plate XI, fig. 2). Front tarsi with eleven white rings or spots on first joint, five on second, four on third and two on fourth and fifth. Third tarsi with ten white spots on first joint, five on second, four on third, three on fourth; fifth white with two narrow black rings.

*Length* 6.5 mm. *Wing* 5.5 mm.

One ♀ taken at La Cabrera, Estado Carabobo, Autumn, 1921, by Dr. M. Nunez Tovar.

This large and beautiful species approaches closely in the markings of the wing to *Anopheles (Arribalzagia) punctimacula*, Dyar and Knab, as described by Howard, Dyar and Knab (1917), under the name *A. malefactor*. The chief differences are tabulated below.



*A. punctimacula*, D. and K.*A. venezuelae*, sp.n.

Length	...	...	...	About 5.0 mm.	...	...	...	6.5 mm.
Wing	...	...	...	About 4.5 mm.	...	...	...	5.5 mm.
Vein III	...	...	...	Two small spots at and near base and two others at and near apex, a few black scales scattered along its whole length.				Base pale, two small black spots near base, two others at and near apex, only one group of two small black scales on rest of upper surface of vein.
Distance between anterior and basal cross veins				About equal to length of basal cross vein				Less than a quarter of the length of the basal cross vein
Hind tarsal segments 3	...	...	...	1 apical, 1 basal, and 1 median, white ring				2 white rings between white apical and basal rings
Hind tarsal segments 4	...	...	...	As third segment	...	...	...	With 1 apical, 1 basal, and 1 median ring
Hind tarsal segments 5	...	...	...	Entirely white (or with a black band)*				With two black bands

\* Dyar, 1918

*Limatus durhamii*, Theo.

La Barraca, near Maracay, ♀ 1.

*Culex quinquefasciatus*, Say.

Houses and buildings in and around Caracas, ♂♂ 275, ♀♀ 738.

Dr. Chacin. About 100 ♂♂ were determined by the genitalia.

Near Maracay, ♂♂ 8, ♀♀ 4. Dr. M. N. Tovar.

*Culex declarator*, D. and K.

Near Maracay, ♂ 1, ♀ 1. Dr. M. N. Tovar.

*Culex corniger*, Theo.

Caracas, ♂ 1, ♀ 1. Dr. Chacin.

*Aedomyia squamipennis*, Theo.

Near Maracay, ♀♀ 2. Dr. M. N. Tovar.

*Mansonia titillans*, Walker.

Near Maracay, ♀♀ 2. Dr. M. N. Tovar.

*Psorophora posticus posticus*, (Wied.) Dyar.

Near Maracay, ♂♂ 2, ♀♀ 5. Dr. M. N. Tovar.

*Psorophora posticatus sayi*, D. and K.

Near Maracay, ♀ 1. Dr. M. N. Tovar.

*Psorophora lutzii*, Theo.

Near Maracay, ♀♀ 2. Dr. M. N. Tovar.

*Psorophora saeva*, D. and K.

Near Maracay, ♀♀ 2. Dr. M. N. Tovar.

The specimens differed from the description of *P. saeva* in the monograph of Howard, Dyar and Knab (1917), in having the scales of the proboscis sub-erect.

*Psorophora ciliata* (Fab.) R.D.

Near Maracay, ♀♀ 2. Dr. M. N. Tovar.

*Psorophora (Janthinosoma) tovari*, sp.n.

FEMALE.

*Proboscis* uniform; labellae small, conical; vestiture of dark scales with violet reflections. Palpi about one-fifth of the length of the proboscis, curved, with partially erected black scales with violet reflections, and a few rather long, coarse setae. Antennae: long segments very dark brown, with delicate white decumbent hairs, setae of whorls blackish brown. Tori ochraceous externally, dark brown internally, dark area with a row of pale flat scales and a number of dark setae. *Clypeus* large, very shining black above, ochraceous at sides above, the colours separated in a distinct line at sides below shading to dark brown. *Eyes* large. *Occiput* wide; integument dark shining blackish-brown above, tawny below. Vestiture of broad very much curved (much more strongly curved than the pale scales on the mesonotum of *P. posticatus*, Wied.) creamy white scales scattered over entire surface, intermixed with creamy upright forked scales on median third, and with broad curved yellowish scales and dark brown setae on lateral thirds; coarse dark setae anteriorly, and a tuft of ochraceous setae projecting between eyes. Posteriorly, upright forked scales black.

*Prothoracic lobes* with silvery, much curved scales, and numerous very coarse, black setae. *Mesonotum*: integument very dark brown, dull. Vestiture on centre of disc of narrow curved, brown scales with brassy reflections, these scales extending laterally behind almost to wing roots.

Sides of disc with broad much curved, creamy scales resembling those on occiput intermixed with a smaller number of bronzy, broad, curved scales. A median band, narrowed behind, of broad, much curved, creamy scales, on anterior sixth. Posteriorly mesonotum partially denuded, antescutellar space dotted with spindle-shaped ochraceous and creamy white scales intermixed. *Pleurae*: integument dark, shining, sepia; above with creamy scales as on sides of mesonotum; below with numerous broad, flat white scales. *Scutellum* with broad, flat, creamy and yellowish white scales. *Postnotum* nude, dark brown, shining.

*Abdomen*. First segment with a median broad patch of creamy white, flat scales, scattered pale ones at sides and numerous fine pale hairs; laterally a conspicuous patch of creamy scales not visible from above. Segments two to six with dark bronzy scales with brilliant metallic blue reflections, and conspicuous apical bands of creamy yellow scales, continuous at sides with pale scales of venter. Segments two and three with creamy band narrowed at sides, and produced backwards medially forming a wide triangle with apex approaching within a third of the base of the segment. On segment four, median backward extension of band broader, truncated. Segments five and six with band broadest in middle, gradually narrowed at sides. Segment seven mostly pale scaled above.

*Venter* entirely clothed with pale golden and silvery scales, but proximal half of second segment denuded.

*Wings*: Membrane deeply infuscated. First fork cell one and a half times the length of its petiole; second fork cell slightly longer than its petiole. Basal cross vein separated from anterior cross vein by nearly its own length. Outstanding scales sepia, ligulate. *Halteres*: knobs brown, stems ochraceous. Metatarsi of hind legs with no sub-erect scales. Front and mid-femora pale straw coloured with dorsal broad stripe and apical narrow ring of bronzy scales with metallic violet reflections. Hind femur with bronzy metallic scales. Tibiae and tarsi clothed with dark bronzy metallic scales, a stripe of scales with ochraceous reflections on under sides of front and middle tibiae, lines of scales with brassy reflections on under sides of the metatarsi and tarsi. Hind legs with segments three, four and five of tarsi missing.

Claws of first and second tarsi with teeth:—I·I—I·I.

Length c. 5 mm. Wing c. 4 mm.

Two ♀♀ taken in region of Maracay, Venezuela. Dr. M. Núñez Tovar, 1921.

*Aedes argenteus*, Poiret.

Houses and buildings in and around Caracas, ♂♂ 28, ♀♀ 327.  
Dr. Chacin. Near Maracay, ♂♂ 18, ♀♀ 6. Dr. M. N. Tovar.

*Aedes trivittatus* (Coq.), D. and K.

Near Maracay, ♀ 1. Dr. M. N. Tovar.

*Aedes (Finlaya) oswaldi*, Lutz.

Near Maracay, ♀ 1. Dr. M. N. Tovar.

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While the present paper was in the press an extensive collection of *Arribalzagia* sp. from the Panama Canal Zone has been obtained. A preliminary examination of these specimens leads me to consider that the characters on which Dyar (1918) defines the species of *Arribalzagia* in his tables may be extremely variable. It seems probable that, although the type of *A. venezuelae* does not fall under any of the species in this table, and does not agree in detail with any of the descriptions of the existing species, it is in reality a variety of *A. punctimacula*, D. and K. A detailed examination of the material is being made, and a further note on the subject will be published shortly.





EXPLANATION OF PLATE XI

*Anopheles (Arribalzagia) venezuelae*, sp.n.

Fig. 1. Wing.

Fig. 2. Last three segments of hind tarsus.

Both figures drawn with camera lucida.

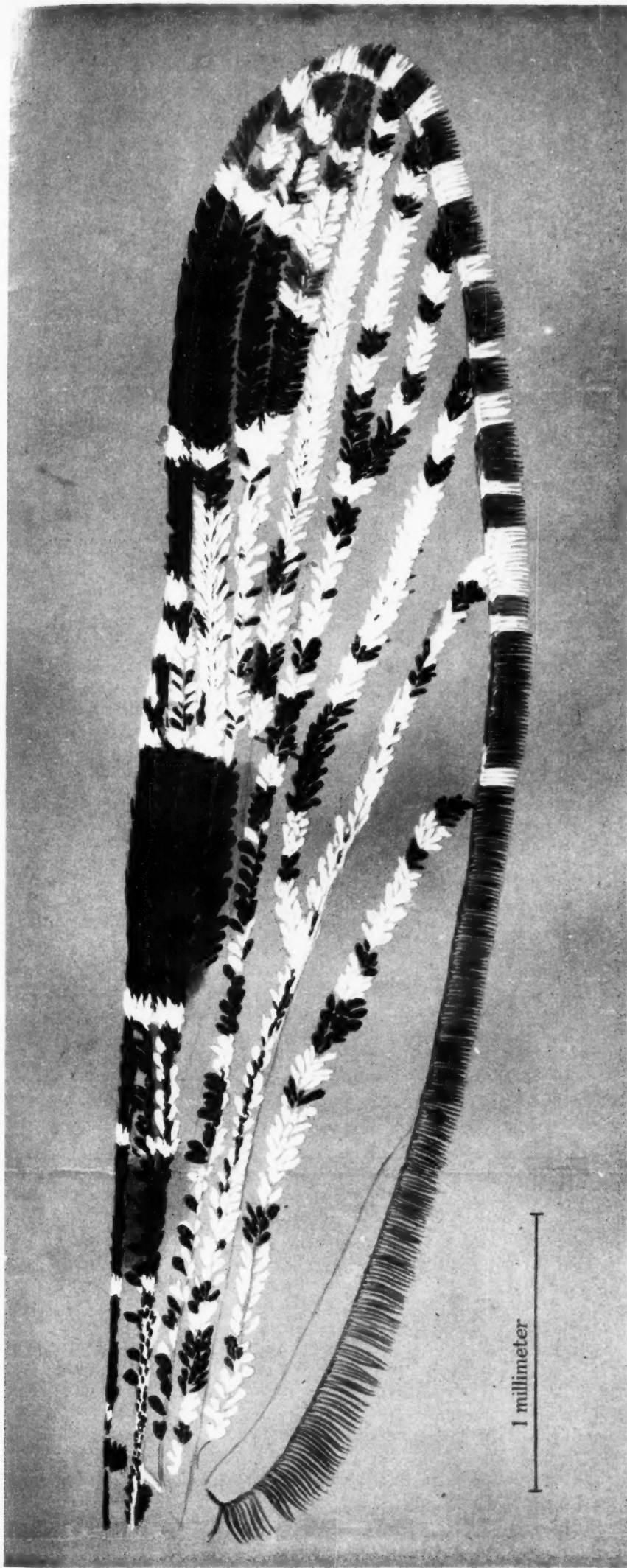


FIG. 1

*A.M.E. del.*

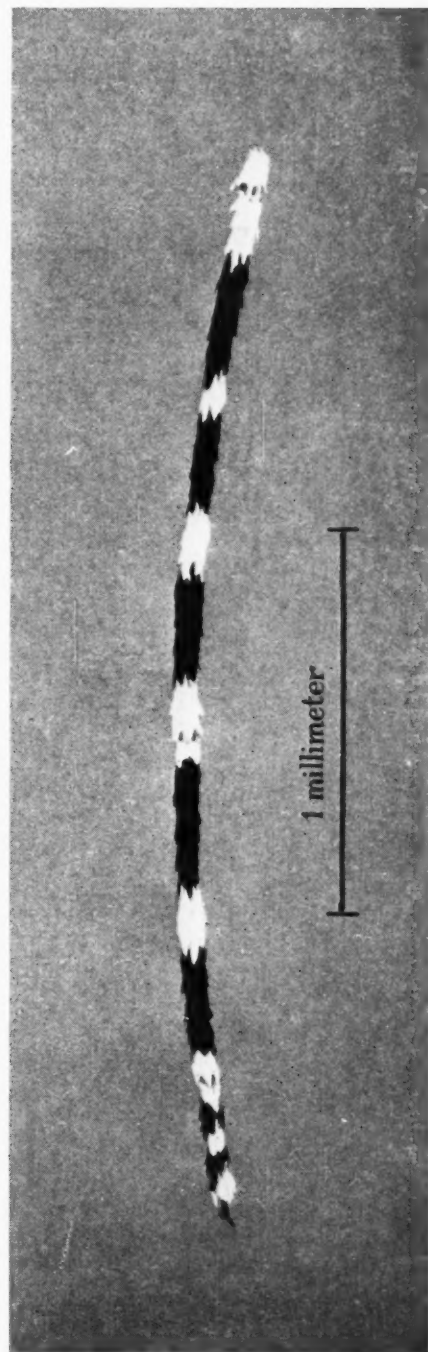


FIG. 2

*C. Tinling & Co., Ltd., Imp.*



# ANCYLOSTOMES RECORDED FROM SIXTY-SEVEN POST-MORTEM PERFORMED IN AMAZONAS

BY

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*(Received for publication 12 June, 1922)*

This paper deals with the ancylostomes collected at sixty-seven autopsies, performed in the Santa Casa Hospital, Manáos, during 1921 and the beginning of 1922. With very few exceptions, all the subjects had resided for the greater part of their lives in the State of Amazonas, Brazil. They divided themselves into two natural classes:—(1) The 'Town-dwellers' and (2) the 'Country-dwellers,' the latter mostly agriculturalists, rubber-workers, etc., who either came into the town to be treated for sickness, or else who were taken ill when temporarily residing there.

**METHOD OF COLLECTION.** The gut having been opened, all ancylostomes, attached or lying loose in the lumen, were removed. The contents of the bowel were then distributed in large, flat, white dishes and examined for ancylostomes during three washings. All worms were washed in normal saline, killed with hot 75 per cent. alcohol, and stored in lacto-phenol (Leiper).

**METHOD OF EXAMINATION.** In the first part of the investigation an attempt was made to estimate the accuracy of a hand-lens ( $\times 8$ ) examination of the worms in order to determine sex and species. For this purpose, the worms obtained from fifteen post-mortems were examined as follows:—First with a hand-lens and a tentative diagnosis made as to sex and species (*i.e.*, whether *Necator americanus* or *A. duodenale*). They were then re-examined with a microscope, using the half-inch and the one-sixth.

The points of distinction noted, during the hand-lens examina-

tion, were (1) the general fineness, and (2) the sharply defined head curve, of *Necator americanus* as compared with *A. duodenale*. In this manner six hundred and sixty-two worms were examined; these consisted of eighty-five *A. duodenale* and five hundred and seventy-seven *Necator americanus*. The result was as follows:—One worm was diagnosed wrongly (*Necator americanus* male, mistaken for *A. duodenale* male); three other worms necessitated microscopical examination, but two of these proved to be so damaged that the head curve was destroyed; the remaining six hundred and fifty-eight worms were found to have been correctly diagnosed with the hand-lens.

With a view to testing whether *A. caninum* or *A. braziliense* could be distinguished from *A. duodenale* and *A. necator*, one male and one female *A. caninum* and one male *A. braziliense* (all from a cat) were mixed with sixty *Necator americanus* and eighteen *A. duodenale*. The worms were then separated into their species by the aid of a hand-lens, the result checked by a microscope and found to be correct.

The distinctions between *A. necator* and *A. caninum* or *A. braziliense* were based on the characteristic head curve of *A. necator*, between *A. duodenale* and *A. caninum* or *A. braziliense* on the smaller size and general fineness of the latter two species.

As this method appeared sufficiently accurate, the worms were sorted with a hand-lens in all subsequent examinations, any doubtful specimens, and these averaged one in sixty, being placed on one side and subsequently examined microscopically.

RESULTS. These are published in the form of a table for comparison with Darling and Smillie's (1921) figures for Brazil. Apparently their results are drawn from Southern Brazil, chiefly from Rio, Pernambuco, Sao Paulo, and a few from the State of Matto Grosso.

They state that 'the groups studied were all more or less similar in that they were composed largely of agriculturists. The average hookworm count of 136.1 per case, therefore, does not represent the degree of infection of *all Brazil*, but of *rural Brazil*.'

As my results are drawn from two classes, a second table is published showing a comparison between town and country infections. It must be noted that a few of the cases recorded had at one



time or another been in hospital, and a certain number of those had undoubtedly received *Chenopodium*.

On examining Table I, it will be seen that the most striking difference between the figures for Amazonas and South Brazil lies in the proportion of *Necator americanus* to *A. duodenale*, and, on examining Table II, that this difference is mainly due to the high average number of *A. duodenale* occurring in the country dwellers. Whereas Darling's rural dwellers for South Brazil show a proportion of *Necator americanus* to *A. duodenale* of 45 to 1, rural dwellers in Amazonas show a proportion of only 3.2 to 1.

*Ancylostoma braziliense* in human beings. Four worms belonging to the species *A. braziliense* were found among the six thousand eight hundred and fifty-seven ancylostomes, collected from the sixty-seven post-mortems. There were two males and two females; the males measured about 7 mm. in length and the females 7.5 mm.

Each worm was found in a separate host. Two were found in native Amazonians who, so far as is known, had never left the State of Amazonas; one in a patient who originally came from Ceara, and one in an American of the 'beach comber' type who had lived some twenty years in North Brazil.

I can find no previous record of *A. braziliense* being found as a human parasite in America. De Faria (1916) states that he examined children in Rio for this infection without success. Darling and Smillie (1921) do not record it among the sixty three thousand nine hundred and twenty-three hookworms they examined in South Brazil; but Darling (1920) writes:—'The ancylostomes encountered in man are *A. duodenale*, *A. ceylanicum*, *A. braziliense*, *Necator americanus*.' I cannot, however, find the authority on which *A. braziliense* is included.

According to de Faria (1910 and 1916) and Clayton Lane (1916), the distinction between *A. braziliense* and *A. ceylanicum* depends on the following two points:—

(1) *The inner ventral tooth.* This is smaller and finer in *A. braziliense* than in *A. ceylanicum*.

(2) *The bursa of the male.* De Faria (1916) states that in *A. braziliense* the rays, especially the dorso-external, are characterised by their great length, fineness and delicacy, whilst those of *A. ceylanicum* are shorter and thicker.

TABLE I

Comparing Ancylostome Infections for Amazonas and South Brazil.

	Amazonas July, 1921 to February, 1922	South Brazil (Darling) April, 1918 to January, 1920
Number of cases examined ... ..	67	469
Number of Ancylostomes found ... ..	6,857	63,923
Number of <i>Necator americanus</i> ... ..	5,660	62,554
Number of <i>A. duodenale</i> ... ..	1,193	1,369
Number of <i>A. braziliense</i> ... ..	4	—
Proportion of <i>Necator americanus</i> to <i>A. duodenale</i> ... ..	4.7 : 1	45 : 1
Average number of Ancylostomes to each individual ... ..	102.3	136.1
Average number of <i>Necator americanus</i> to each individual ... ..	84.4	133.2
Average number of <i>A. duodenale</i> to each individual ... ..	17.8	2.9

TABLE II.

Comparing Ancylostome Infection of Country and Town Dwellers in Amazonas.

	Country Dwellers	Town Dwellers
Number of cases examined ... ..	39	28
Number of Ancylostomes examined ... ..	4,144	2,713
Number of <i>Necator americanus</i> ... ..	3,157	2,503
Number of <i>A. duodenale</i> ... ..	985	208
Number of <i>A. braziliense</i> ... ..	2	2
Proportion of <i>Necator americanus</i> to <i>A. duodenale</i> ... ..	3.2 : 1	12 : 1
Average number of Ancylostomes to each individual ... ..	106.2	96.8
Average number of <i>Necator americanus</i> to each individual ... ..	80.9	89.3
Average number of <i>A. duodenale</i> to each individual ... ..	25.2	7.4

The distinction between the two was disputed by Leiper (1913). I have had the opportunity of comparing the following ancylostomes :—

- (1) *A. braziliense* from cats and dogs in N. Brazil.
- (2) *A. braziliense* from human subjects in N. Brazil.
- (3) *A. ceylanicum* from cats and dogs in Bengal, India.  
(Material kindly supplied by Lt.-Col. Clayton Lane.)
- (4) *A. ceylanicum* from West African dogs and South African cats.

As a result of careful examination of many specimens, I was unable to confirm the specific differences mentioned by de Faria and Clayton Lane.

No constant difference could be detected in the size and shape of the inner tooth of *A. braziliense* and *A. ceylanicum*, nor could any difference be discovered in the length and fineness of the dorso-external ray in the two worms (*vide* table).

TABLE III.

Comparing Measurements of the Dorso-external Ray in *A. ceylanicum* and *A. braziliense*

As named	Locality	Host	Number examined	Average length of worm	Average breadth D.E.R.	Average length D.E.R.	Ratio length D.E.R. to length worm	Ratio breadth D.E.R. to length worm
<i>A. ceylanicum</i>	Berhampore, Bengal	Cat ...	3	mm. 5.6	$\mu$ 14	$\mu$ 176	1 : 31	1 : 400
<i>A. ceylanicum</i>	Berhampore, Bengal	Dog ...	1	7.5	21	217	1 : 34	1 : 357
<i>A. ceylanicum</i>	Accra, West Africa	Dog ...	3	7.0	20	270	1 : 25	1 : 350
<i>A. braziliense</i>	Manáos, North Brazil	Dog ...	4	6.5	17	171	1 : 38	1 : 382
<i>A. braziliense</i>	Manáos, North Brazil	Cat ...	3	6.1	17	186	1 : 32	1 : 358
<i>A. braziliense</i>	Manáos, North Brazil	Human	2	7.0	14	162	1 : 43	1 : 500

## SUMMARY

Six thousand eight hundred and fifty-seven ancylostomes collected from sixty-seven autopsies performed in Manáos, Amazonas, were examined, with the results recorded. A far higher proportion of *A. duodenale* to *Necator americanus* (1 : 4·7) occurred in Amazonas than recorded by Darling for South Brazil (1 : 45). This high proportion of *A. duodenale* was shown to be chiefly due to the country dwellers in Amazonas, whose *A. duodenale* to *Necator americanus* ratio was 1 : 3·2, while that of the city dweller was 1 : 12.

*A. braziliense* was found in four of the post-mortems.

The comparison of these worms and other two-toothed ancylostomes from dogs and cats in N. Brazil and India, and also from cats in South Africa and dogs in West Africa, failed to show the difference claimed to exist by de Faria between *A. ceylanicum* and *A. braziliense*.

My thanks are due to Dr. Thomas for much of the post-mortem material.

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# THE SUSCEPTIBILITY OF THE INDIVIDUAL TO THE BITES OF *STEGOMYIA CALOPUS*

BY

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*(Received for publication 27 May, 1922)*

The usual belief amongst Europeans residing in the Tropics, with regard to the susceptibility of the individual to the biting of mosquitoes, would appear to be that the new-comer receives proportionately more bites than the old resident, but that the native of the country receives less than either.

Marchoux, Salimbeni and Simond (1903), writing of *Stegomyia calopus*, state ' . . . Il a une prédilection marquée pour la race blanche.' And later in the same article, ' Il s'attaque beaucoup plus avidement aux individus jeunes, vigoureux, qui ont la peau fine et le teint coloré, qu'aux individus anémiés ou âgés.'

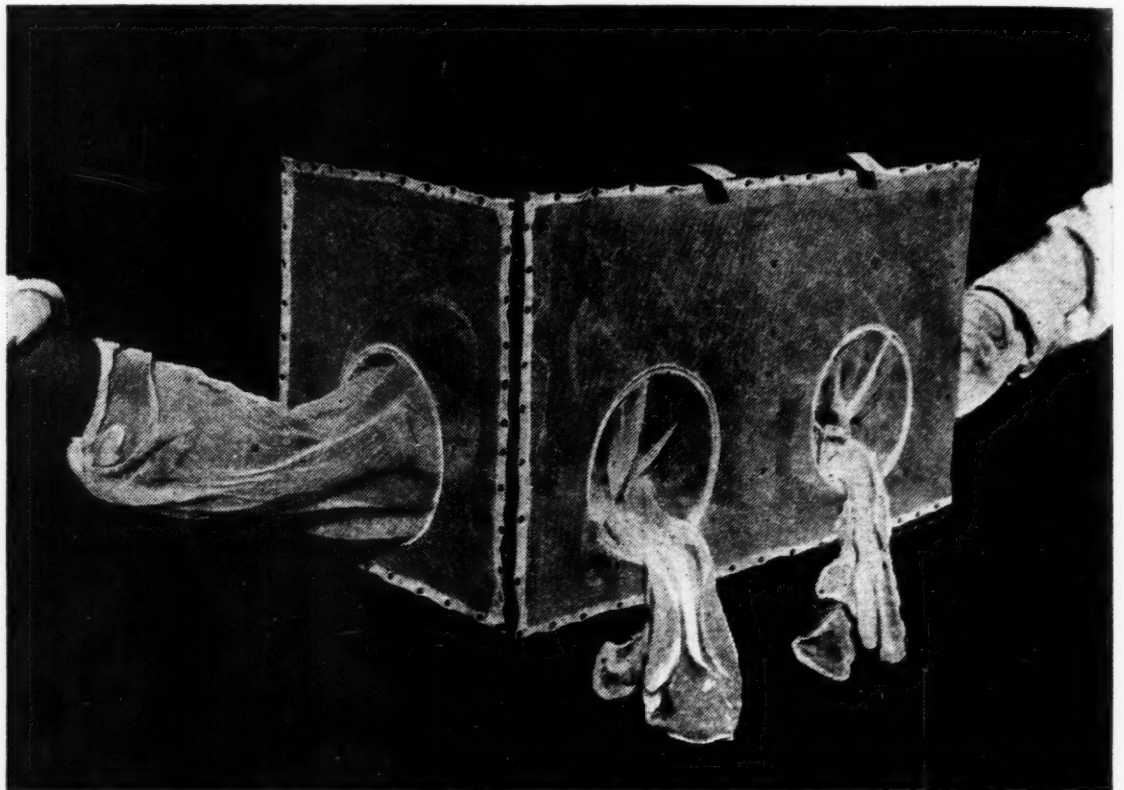
It appeared of interest to test the truth of this idea and, at the same time, to investigate the following points with regard to their influence on the biting of mosquitoes: (1) Sweating; (2) hairiness of skin exposed to bites; (3) colouration; (4) age. Attention was also paid to the subsequent local reaction to the bites.

*Nature of Experiment.* All experiments were performed with *Stegomyia calopus*, owing to its being a day-feeder and the commonest mosquito in the locality.

Sixteen experiments were performed, at each of which a number of male persons, usually six, of various nationalities and different lengths of residence in Brazil, were exposed, under the same conditions, to the bites of a number (usually forty-five to fifty) of

hungry *Stegomyia calopus* females. The number of completed feeds performed on each individual during 30 minutes were noted.

*Apparatus used and Method of Recording Results.* The feeding-box consisted of a large mosquito cage measuring 24 by 15 by 12½ inches, and fitted with six sleeves. All experiments were performed in daylight at approximately the same hour, and the box placed in such a position that it was as far as possible evenly illuminated.



Mosquito cage used for the experiment. In practice, the sleeve was fitted closely to the forearm, at its junction with the cage wall.

Forty to fifty female mosquitoes, which had been kept unfed for at least four days since their date of emergence, were released in the cage. The individuals to be tested then introduced one of their hands through the sleeves so that each had the same amount of forearm and hand exposed to bites.

It was found in practice that the female *Stegomyia*, unless disturbed, never bit twice, and counts could be made easily and accurately.

After the first two experiments it was noted that mosquitoes that bit on the under surface of the wrist and hand were hard to count, and, at Dr. H. Wolferstan Thomas's suggestion, in all subsequent experiments, cardboard shields were used to protect this surface.

An example of an experiment is given to show the data recorded.

EXPERIMENT 3. Date: 3.10.21. Number: *Stegomyia calopus* = 50.

Name	Age	Colouration	Hairiness of exposed arm	Nationality	Number of years in Brazil	Residence in other countries with service in each	Sweating : first 15 mins.	Sweating : second 15 mins.	Number of bites received
O.K. ...	30	Dark	○	British	9	England only	+	+	8
M.L. ...	35	Dark	○	Portuguese	16	Portugal 20 years	+	+	13
B.E. ...	25	Fair	○	British	2	England and Canada only	○	+	7
M.S. ...	20	Dark	○	Brazilian	18	Nil	○	○	8
T.S. ...	47	Fair	+	Canadian	15	England and Canada only	○	+	3
L.O. ...	23	Dark	+	Brazilian	23	Nil	+	+	9

#### RESULTS.

##### I. Length of Residence.

	Total number of individuals tested	Total number of bites received	Average number of bites received by each individual
Persons above 5 years' but under 30 years' residence in Brazil. (Majority 10-15 years) ... ..	62	462	7.4
Persons under 2 years' residence in Brazil. (Majority under 1 year, some a few weeks) ... ..	26	157	6.0

## II. Sweating.

	Total number of individuals tested	Total number of bites received	Average number of bites received by each individual
Persons sweating on the exposed forearm and hand ...	30	212	7.0
Persons not sweating on the exposed forearm and hand	58	407	7.0

## III. Hairiness.

	Total number of individuals tested	Total number of bites received	Average number of bites received by each individual
Persons showing a considerable amount of 'hairiness' on the exposed forearm and hand ... ..	40	286	7.1
Persons not showing any marked 'hairiness' of exposed forearm and hand ... ..	48	333	6.9

## IV. Colouration.

	Total number of individuals tested	Total number of bites received	Average number of bites received by each individual
Persons of a dark colouration ('dark' being used in the accepted sense of dark eyes and hair) ... ..	62	441	7.1
Persons of a fair colouration ... ..	26	178	6.8



## V. Age.

	Total number of individuals tested	Total number of bites received	Average number of bites received by each individual
Persons of 30 years and under ... ..	29	194	6.6
Persons of more than 30, and less than 40 ... ..	36	277	7.6
Persons of more than 40 ... ..	23	148	6.4

## VI. Nationality.

	Total number of individuals tested	Total number of bites received	Average number of bites received by each individual
Persons of British, Canadian or American extraction ...	57	352	6.1
Persons of Portuguese extraction ... ..	23	193	8.3
Persons of Brazilian extraction ... ..	6	59	9.8
Persons of Chinese extraction ... ..	1	7	7.0
Persons of African native extraction ... ..	1	8	8.0

*Local Reaction.* It was found impossible to record this in figures for lack of standard comparisons, but the impression of all observers during the experiment was that, as a class, the new-comers reacted most, the long-resident Europeans less and the native Brazilians least; in the case of the last named, as a rule, no reaction whatsoever could be detected.

## SUMMARY

Before any conclusions can be drawn from these figures, two fallacies must be considered:—

(1) To obtain true results each of the sixteen experiments should be considered on its own merits, but this would demand too much space; as, however, it was arranged that as far as possible at each experiment approximately the same proportion of variable factors (*i.e.*, hairiness, nationality, etc.) should be present, and as the proportion of sweating to non-sweating individuals remained nearly constant, it appeared legitimate to add together the number of bites received in the sixteen experiments.

(2) The number of individuals tested and the number of bites recorded are so small that no definite conclusions can be drawn; they merely *suggest* what follows:—

Eighty-eight male persons of various nationalities and ages were tested with regard to their susceptibility to the bites of *Stegomyia calopus*. Six hundred and nineteen bites were received in all. The following factors were recorded:—

(1) Length of residence in Brazil; (2) sweating of surfaces exposed to bites; (3) hairiness of skin exposed to bites; (4) colouration; (5) age; (6) nationality.

The resulting figures would seem to show that none of these factors exert any marked influence on the number of bites received by the individual.

The theory that the number of mosquito bites received by the new-comer is greater than those received by the old resident, both being greater than those received by the native of the country, would appear to be, in part at any rate, attributable to the local reaction immunity displayed by the native, and to a less extent by the old resident.

I am indebted to Dr. H. W. Thomas for much help and suggestion.

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# TUBERCULOSIS IN THE SUDAN, WITH NOTES ON A CASE OF BREAST TUBER- CULOSIS IN A SUDANESE

BY

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*(Received for publication 17 June, 1922)*

## PLATE XII

It would appear from the number of papers recently published dealing with the subject of 'Tuberculosis in the Tropics' that interest in this disease has been awakened, and in view of its importance, the writer feels little apology is needed for offering some general observations on 'Tuberculosis in the Sudan.'

Unfortunately, such observations can in no sense be considered complete, as they are based on a limited amount of clinical and pathological material obtained during the past fourteen years from almost every district of the Sudan. Limited though this material has been, it is in the main representative of a disease which, happily, has not assumed the proportions prevailing in Western countries or even in some of the towns of the Far East.

The reason for this is not far to seek. At the present time the Sudan lacks the thickly populated centres of other countries in the West and East. Vast tracts of desert waste and swamp still await to be linked up by means of railways, and although inter-tribal trade and communications have been encouraged under British administration, there still exists in certain parts a conservatism fostered by racial and religious differences, which will take years to break down.

Once these obstacles are removed, the seeds of tubercle will assuredly grow and be disseminated on what can only be considered a virgin soil.

Any opinion offered as to how and when the disease was first introduced into the Sudan can merely be hypothetical.

In the days of Mahdism and up to the time of the British occupation, the country certainly enjoyed a comparative seclusion from the tide of civilization. On the Northern frontier, little inter-communication occurred with Egypt. The perils attendant on the long desert wastes of this region acted as a deterrent to intimate relations between the two countries, and it is safe to infer that little encouragement was offered to the pagan tribes of the South and West, while on the Eastern Abyssinian frontier, the racial and religious differences of the two countries were sufficient reasons for keeping them aloof.

It is, however, to the earlier history of the country that one must turn for information regarding the introduction of tuberculosis; this history, as will be seen from archaeological records, is intimately connected with the ancient history of Egypt.

As far back as 2600 B.C. the Northern Sudan was invaded by the Egyptians, and from 2000-1000 B.C. this portion of the Sudan appears to have been occupied by them and regular colonies established as far south as Kerma in the Dongola Province.

That tuberculosis was existent among the Egyptians during these periods was established by the late Sir Armand Ruffer (1921), whose work on the 'Palaeopathology of Egypt' is well known. In an admirable collection of his studies on the subject, edited by Prof. Moodie, of Illinois University, there are plates illustrating Pott's disease in figures discovered in the tombs of Beni Hassan, 2000 B.C. Two other plates also depict graphically Pott's disease, and a large psoas abscess in a mummy of a priest of Ammon of the XXIst Dynasty, 1000 B.C.

Derry's (1907-08) investigations recorded in the 'Archaeological Survey of Nubia,' apart from representing the first record of tuberculosis in the Sudan, afford circumstantial proof of the introduction of the disease from Egypt into Lower Nubia, and it appears reasonable, therefore, to infer that tuberculosis obtained a footing in the Sudan synchronously with the tide of settlers from Egypt, but did not spread throughout the country for reasons already mentioned.

At the present time there is little doubt that the disease is practically confined to the larger towns of the Northern Sudan.

It is here that the factors concerned with stress, and resultant to a great extent on civilization, play no small part. Overcrowding,

intestinal parasitism, malaria, venereal disease, alcoholism and the 'hasheesh habit' undoubtedly predispose to infection by lowering the resistance of individuals peculiarly susceptible to the virus of tubercle.

Amongst the hardy, simple-living nomadic tribes of the desert and the comparatively secluded tribes of the South, the disease is practically unknown; with increasing facilities for inter-communication, however, such a state of things is unhappily not likely to continue.

For obvious reasons, more especially when one is dealing with a Mahomedan population, it is impossible to obtain statistics regarding the incidence of, or death rate from, tuberculosis. Racial and religious prejudices often interfere with the calling in of qualified medical aid, and post-mortems are rarely obtained except in cases presenting a medico-legal aspect. Consequently one is compelled to admit that figures obtained from hospitals and dispensaries do not represent the true incidence of tuberculosis in the country, and the writer is of the opinion that such incidence is higher than is suspected. Unfortunately von Pirquet's test has not been carried out on a sufficiently large scale to permit of any deductions being made.

The tribes of the Sudan are very susceptible to such respiratory diseases as bronchitis, broncho-pneumonia and pneumonia, and their predisposition to tuberculosis was referred to many years ago by Balfour (1904). 'The Sudanese or 'black' appears peculiarly susceptible, and it is stated that the Hadendowa, a black tribe inhabiting the hills of the Red Sea, shares this susceptibility. Other observers, notably Bushnell (1920) and Cummins (1920), have called attention to the susceptibility of coloured races to tuberculosis.

Moreau (1919) and Roubier (1920) have pointed out the difficulties in detecting the disease among black troops even when the patients are greatly infected, and they prove the value of radiological examination in such cases.

The same difficulties are experienced in the Sudan, especially as regards pulmonary tuberculosis, and it may not be amiss to mention here that the disease is at times simulated by bronchial spirochaetosis and a bronchitis of streptococcal origin.

The predisposing causes to tuberculosis in the Sudan have already been referred to, and there is no doubt that overcrowding



and the filthy habit of expectoration are the determining factors concerned with the spread of the disease, more especially in the cold winter months when overcrowding to the exclusion of light and air favour the possibilities of 'massed infection.' Scott's (1921) observations equally emphasize the rôle played by overcrowding and expectoration as causative factors in tuberculosis among the Chinese in Hong Kong.

The view that infected milk is a cause of tuberculosis in the Sudan may readily be dismissed; it is true that goats' milk, cows' milk, and to a less extent camels' milk, represent an important feature in the dietary of the natives of the country; nevertheless, tubercular disease of these animals is unknown. Many years ago the writer (1910) recorded a case in which acid-fast bacilli were found in lesions of the lung of a camel simulating miliary tuberculosis, but it should be stated that the possibility of these lesions being caused by an organism of the streptothrix or *nocardia* group could not be excluded.

From the evidence obtained it would appear that inhalation is the common method of infection, such infection arising from dust-infected particles. Once tuberculosis is established in the lung, dust appears to be an irritating factor favouring the progress of the disease; incidentally it may be mentioned here that the practice of recommending cases of early tuberculosis to a country such as the Sudan is one to be deprecated inasmuch as they invariably become worse.

With regard to sex and age, the disease appears to be more prevalent among adult males, but allowance should be made for the fact that racial customs, more especially in some parts of the Sudan, do not encourage the female population to seek medical advice; however, having due regard to this, it would appear that the disease is more prevalent among the itinerant male population, a fact which is not in accordance with Lankester's (1920) observations in India. The children of the Sudanese appear to be rarely affected.

Of the varieties of tubercular disease in the Sudan, adenitis is perhaps the commonest; with lung tuberculosis, and a pleurisy of tubercular origin next in frequency; general miliary tuberculosis also occurs probably more commonly than is suspected, presenting with its pyrexia, cachexia, and splenomegaly, a clinical picture often

difficult of diagnosis and readily confused with other diseases. Pott's disease is exceedingly rare, and tubercular meningitis more so. It is doubtful whether skin tuberculosis exists. Cases labelled as such have, on bacteriological examination, proved to be early tubercular leprosy.

A few cases of joint tuberculosis have been observed by the writer, but are uncommon. Intestinal tuberculosis occurring as a primary affection of the intestines is exceedingly rare, as would be expected in a country where animal tuberculosis is non-existent.

Recently a case of breast tuberculosis came under the writer's observations, and as the disease is of sufficient rarity even in Western countries, a few detailed notes regarding this case are appended.

The patient was a Sudanese woman, about 40 years of age, hailing from the remote hilly districts of Kordofan, where she had spent the greater part of her life. She was married, and had a grown-up daughter who was in good health. According to her statement, her illness commenced some sixteen months ago with a painful swelling of the breast, which was not attributed to any injury received. The symptoms lasted for a period of twelve months and then subsided; however, about three months ago, she had recurring attacks of pain, and decided to come to Khartoum for treatment.

On admission to hospital her general condition was good, and during the few days prior to operation she showed a slight rise of temperature in the evenings.

On examination of the affected left breast, there was apparent a marked retraction of the nipple (Plate XII, fig. 1), but no evidence of ulceration or scar formation. On palpation, a nodular condition of the breast was detected. The nodules appeared to be located in the breast substance, were firm in consistency, and freely movable over the subjacent muscle tissue. The axillary lymphatic glands on the left side showed no appreciable enlargement, and were painless on palpation. The right breast appeared to be perfectly healthy.

Apart from the breast pain, the patient complained of no other symptoms. Examination of the lungs, heart and abdominal viscera revealed no abnormalities, nor were any enlargements of the cervical, subclavicular, mesenteric or groin glands detected. A total excision of the left breast was carried out, and some enlarged lymphatic nodes encountered during the operation were cleared away.

On sectioning the breast, numerous greyish-white, irregular-shaped nodules of various sizes were found scattered throughout the breast tissue (Plate XII, figs. 2 and 3). At the base of the nipple many of these nodules had coalesced and appeared to be fibrous. The majority of the nodules were firm in consistency; some, on the other hand, had broken down to form soft caseating masses, which could be readily shelled out of a capsule composed of dense fibrous tissue.

Subsequent histological examination of some of these nodules revealed their lymphatic structure.

The gross pathological appearances of the breast suggested tuberculosis in which fibrosis was a marked feature.

Film preparations of the broken down connecting débris were stained for the purpose of demonstrating tubercle bacilli, but with negative results.

Portions of the nodules with adjacent breast tissue were excised, fixed, embedded and sectioned for histological examination. Sections showed almost a complete absence of normal breast tissue. Necrotic foci of various sizes composed of granular amorphous material in which only a few nucleated cellular elements could be seen were scattered throughout the section. The larger foci, representing advanced caseous degeneration, were sharply demarcated by a zone of dense fibrous tissue. The smaller foci showed a pericellular reaction composed chiefly of lymphocytes and connective tissue cells, while scattered irregularly throughout the tissue were giant cells of Langerhans, containing six or more nuclei (Plate XII, fig. 4).

The blood vessels showed a periarteritis and also some thickening of the tunica media.

Sections of the lymphatic nodules showed well marked caseation with separative fibrotic changes and typical giant cell systems. The vessels here also showed a periarteritis and mesarteritis. Sections of the nodules were also stained by special methods to demonstrate tubercle bacilli, but with negative results.

#### REMARKS

There is little or no doubt that the case represented one of tuberculosis of the breast in which reparative changes of a fibrotic nature were a feature. Such changes probably accounted for tubercle

bacilli not being found in the sections and film preparations, and were also responsible for the marked retraction of the nipple.

It is to be regretted that no inoculation experiments were carried out, but in view of the reparative changes noted it is doubtful whether they would have led to a successful issue.

In all probability, the breast was secondarily infected via the lymphatics, although no primary focus of infection could be detected.

In view of its rarity, even in Western countries, the case appears worthy of record, and no similar case appears to have been previously reported from the Sudan.

I am indebted to Dr. Hodson, M.V.O., Director, Khartoum and Omdurman Civil Hospitals, for furnishing the clinical notes of the case and for providing the material for examination.

KHARTOUM,

June 1, 1922.

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## EXPLANATION OF PLATE XII

- Fig. 1. Anterior view of the affected breast showing in the centre the marked retraction of the nipple.
- Fig. 2. Section of the same breast showing the tubercular nodules demarcated by fibrous tissue.
- Fig. 3. Showing a large caseating lymphatic node at the breast margin.
- Fig. 4. Microphotograph of a section showing a single tubercle. In the centre is a giant cell sending protoplasmic processes into the surrounding epithelioid cells. The marginal portion of the tubercle shows the lymphoid cell infiltration.  $\times 170$ .



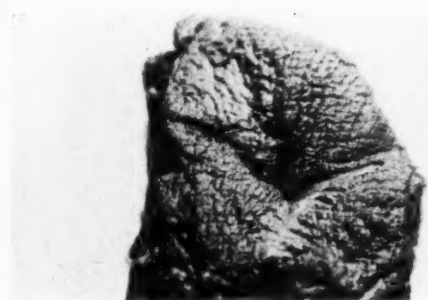


FIG. 1

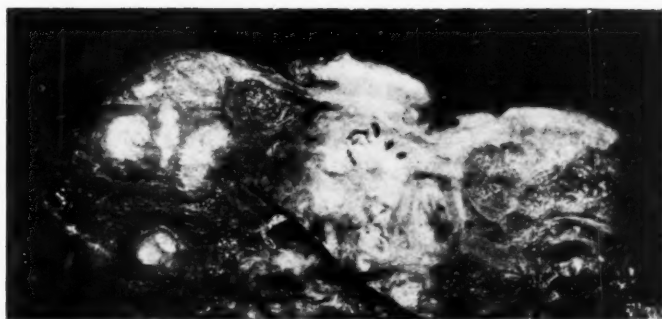


FIG. 2



FIG. 3

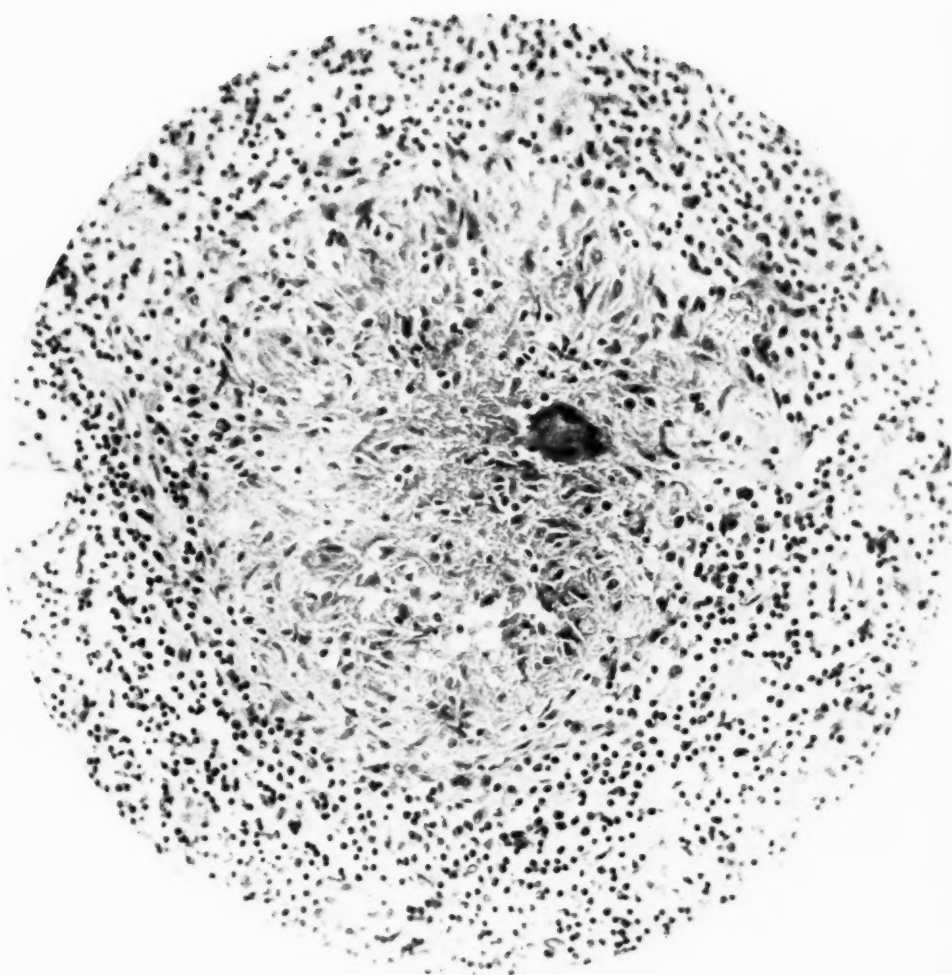


FIG. 4.



## WEST AFRICAN CERATOPOGONINAE

## PART II

BY

A. INGRAM

AND

J. W. S. MACFIE

*(Received for publication 28 June, 1922)*

The majority of the species described in this paper were collected at or near Accra, in the Gold Coast. A few, however, were sent to us from Nigeria, and for these we have to thank Dr. E. C. Braithwaite, of Calabar, and, once again, Dr. H. Andrew Foy, of Lagos.

With regard to the species which in this and our previous papers we have assigned to the Genus *Atrichopogon*, it should be noted that the eyes are not bare. In his paper on the 'Chironomidae of America' (1917), Kieffer associated his Genus *Kempia* with *Atrichopogon*, separating it by the pubescence of the eyes. Later (1921), in a brief note, the same author erected the new Genus *Gymnohelea*, the characters of which also agree with those of *Atrichopogon* excepting that the eyes are pubescent. Kieffer appears therefore, to recognise two genera (*Kempia* and *Gymnohelea*) closely allied to *Atrichopogon* but differing from it in having the eyes pubescent, but he has not stated what are the differences between them, nor indeed, so far as we can ascertain, has he fully detailed the generic characters of either. In the species which we have described, we have found every gradation between those in which the eyes are practically bare and those in which they are densely hairy. In the former, the pubescence may be restricted to the anterior margins of the middle thirds of the eyes and may be visible clearly only after treatment with caustic potash, so that it might be overlooked (as was done by us in some cases) unless, by rolling the specimen from side to side as is possible by our carbolic

technique, the whole eye were carefully examined.\* In our opinion, the hairiness of the eyes cannot, therefore, be considered of more than specific value, and accordingly we have referred our species to the Genus *Atrichopogon*. The species which on a previous occasion (1921) we described as *Kempia ochrosoma* should also, we consider, be referred to this genus.

The figures illustrating the specific descriptions are in most cases mere outlines, drawn with the aid of a camera lucida, omitting such structures as the hairs on the hypopygium, and the fringe and the stronger setae on the costa and basal veins of the wings. The unit of measurement referred to is  $3.8\mu$ .

The types and co-types of the new species described have been deposited in the Museum of the Liverpool School of Tropical Medicine.

*Thysanognathus*† (*Prionognathus*) *albopictus*, sp. nov.

MEASUREMENTS.	Male.	Female.
Length of body‡ (one male and one female) ...	1.2 mm.	1.3 mm.
Length of wing ... ..	0.9 mm.	0.9 mm.
Greatest breadth of wing ... ..	0.23 mm.	0.4 mm.

*Head* dark brown. Eyes narrowly separated above in the female, more widely in the male; in both sexes the space between them wedge-shaped, broadest at the vertex. Clypeus and proboscis dark brown. Palpi dark brown: in the female, the second and fourth segments sub-equal but the fourth the more slender, third rather longer, slightly inflated, with a large pit containing very long sensory hairs, fifth longer than the third, slightly dilated at its end; in the male, second, third, and fourth sub-equal, third not inflated, fifth longer and slightly dilated at its end. *Antennae*: in the female, first segment brown, bearing a few hairs, torus brown, rounded, bearing a few hairs; flagellum pale brown basally and darker brown apically, its segments somewhat flask-shaped, all

\* Referring to this question Mr. F. W. Edwards has written to us as follows: '*Ceratopogon fuscus*, Coq., which I believe is the type of *Atrichopogon*, certainly has the eyes entirely bare, but like you, I have found species of this group which have the eyes only very slightly hairy on the upper part, and bare below. In consequence of this, I have long been of the opinion that *Atrichopogon* and *Kempia* ought to be united.'

† Mr. F. W. Edwards has kindly informed us that a new name is required for this genus as *Prionognathus*, C. I. and M. is preoccupied. See Scudder's *Nomenclator*.

‡ In all cases this measurement is taken from the anterior margin of the thorax to the tip of the abdomen of specimens mounted in carbolic.

about twice as long as broad, and forming a continuous series from base to apex, the last segment being slightly longer and broader, not flask-shaped, ending bluntly without a stylet. In the male, first segment a mere ring of chitin; torus brown, large, bearing a few hairs; flagellum pale brown basally, bearing a pale brown plume, and dark brown distally, the twelfth segment slightly produced distally, length about twice the breadth, the last three segments elongated, nearly five times as long as broad, the last segment ending bluntly without a stylet. *Thorax* dark brown with pale, almost silvery, markings. *Dorsum* dark brown, with a broad median pale stripe, and on each side of it two small pale spots anteriorly and a larger, more diffuse pale area at the root of the wing. These pale markings are larger and more distinct in the male than they are in the female. *Pleurae* brown. *Scutellum* greyish-brown, containing an almost white pigment, bearing in both sexes four central bristles, two anterior and two marginal, the latter close together. *Post-scutellum* dark brown with two large pale, grey, patches anteriorly. *Wings* (fig. 1) hyaline, with two small, blackish spots, one covering

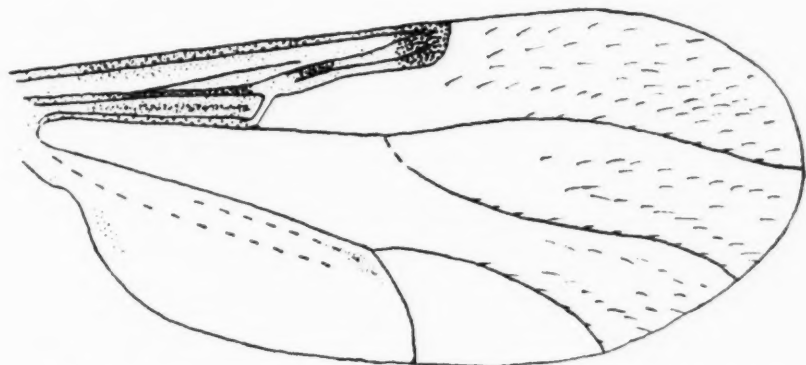


FIG. 1. *Thysanognathus* (*Prionognathus*) *albopictus*, sp.n., wing of female to show distribution of decumbent hairs, adornment, and venation.  $\times$  c. 90.

the extremities of the costa and first and third veins, the other smaller about the middle of the fused portion of the first and third veins, covering only the anterior half of the vein. The anterior half of the basal portion of the wing proximal to the anterior cross-vein is also infuscated. These dark markings are much paler and less distinct in the male. Decumbent hairs fairly numerous in the female on the distal third of the wing at the tip, between the ramus of the fourth vein, and (to a lesser extent) between the fourth and fifth veins; in the male they are entirely wanting. Halteres with greyish-brown,



almost white, knobs, paler in the male than in the female. The knobs contain a whiteish pigment similar to that present in the scutellum and in the abdomen. *Legs* in the female darkish brown, with pale bands; femora with pale sub-apical bands, tibiae with pale sub-basal bands, and on the middle and hind legs pale sub-apical bands also, first three tarsal segments pale, with slightly infuscated apices excepting the first tarsal segment of the hind legs which is entirely dark brown, fourth and fifth tarsal segments of all legs infuscated; in the male the legs are much paler, but similarly marked. Claws in the female unequal, one very large, about as long as the fifth tarsal segment, the other small, on the middle and hind legs about a quarter the length of the segment, on the fore legs longer, about half the length; in the male, claws equal, small, less than half the length of the fifth tarsal segment, with bifid tips. *Abdomen* dark brown with pale grey, almost silvery, markings on the sides and posterior margins of the segments. In the female the pale markings are somewhat broken up into small spots, and are most conspicuous on the fourth to the sixth segments; the tip of the abdomen is white. In the male the pale markings are larger or smaller marginal patches on each side of the middle line, and are most conspicuous on the fifth to the seventh segments; the tip of the body (excluding the claspers) is dark brown. Spermathecae two, highly chitinised, more or less pyriform and slightly unequal; in one specimen the lengths and breadths were about  $80\mu$  by  $75\mu$ , and  $65\mu$  by  $68\mu$  respectively. The chitinised parts of the ducts in the same specimen measured about  $10\mu$  and  $8\mu$  respectively, but as they merged insensibly with the bodies of the spermathecae the measurements are not exact ones.

**HYPOPYGIUM** (fig. 2). Highly chitinised, dark brown, excepting the claspers and the posterior end of the ninth tergite. *Ninth segment*: tergite long, broad and highly chitinised at the base, narrow and feebly chitinised at the apex, very sparsely clothed with hairs dorsally, the posterior margin straight, without either notch or lateral finger-like processes; sternite very deeply and widely excavated. *Forceps*: side-pieces well developed, highly chitinised, tapering distally; claspers very feebly chitinised and very pale coloured, curved slightly inwards, covered all over with minute hairs, and with the ends divided into two small processes, the ventral

one the larger and spine-like. *Harpes* very highly chitinised, distal portion directed posteriorly, broad at the base, tapering towards the

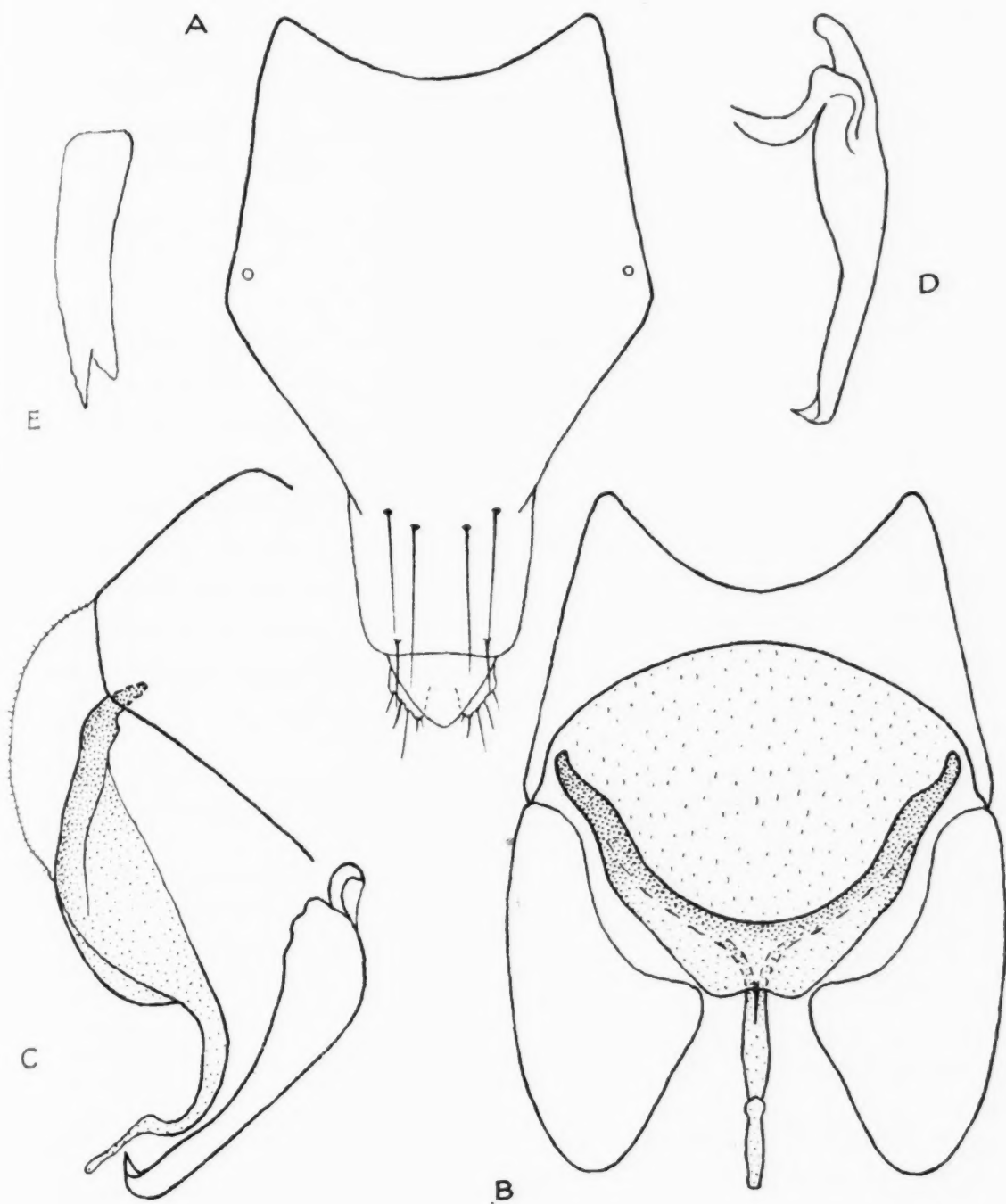


FIG. 2. *Thysanognathus* (*Prionognathus*) *albopictus*, sp.n., male hypopygium. *a*—ninth tergite, dorsal view; *b*—ninth sternite, side pieces, and aedoeagus, ventral view; *c*—aedoeagus and harpe, lateral view; *d*—harpe, ventral view; *e*—clasper, lateral view. All  $\times$  c. 375.

apex, and ending in a short hook. *Aedoeagus* with a very wide basal arch and a long posterior process which is bent sharply at its

end in a ventral direction. Membrane joining the aedoeagus to the ninth sternite covered all over with spicules.

GOLD COAST: Dodowah, 18th February, 1922; two females and one male, reared from material taken from a rot-hole in a mango tree.

*Thysanognathus (Prionognathus) melanostictus*, sp. nov.

MEASUREMENTS.

Length of body (one male)	...	...	...	...	...	1.0 mm.
Length of wing	...	...	...	...	...	0.9 mm.
Greatest breadth of wing	...	...	...	...	...	0.3 mm.

*Head* brown. Eyes separated, the space between them being wedge-shaped, broadest at the vertex. Clypeus, proboscis, and palpi brown. Second, third, and fourth palpal segments sub-equal, fifth longer, slightly dilated distally. *Antennae*: torus very large, brown; flagellum unfortunately missing. *Thorax* greyish-brown with small dark brown spots. Scutellum greyish-brown, dark brown mesially, bearing four central bristles, two anterior and two marginal, the latter close together. Post-scutellum dark brown with two large pale grey areas anteriorly. Pleurae brown. *Wings* hyaline with small blackish spots as shown in the figure (see fig. 3). Decumbent



FIG. 3. *Thysanognathus (Prionognathus) melanostictus*, sp.n., wing of male to show adornment and venation.  $\times$  c. 90.

hairs practically absent, only one or two being present at the periphery near the tips of the wings. Halteres with greyish-brown, almost white, knobs. *Legs* greyish-brown with dark markings similar to those of *P. marmoratus*. Claws similar to those of *P. marmoratus*. *Abdomen* dark brown with pale grey markings.

**HYPOPYGIUM** (fig. 4). Somewhat similar to that of *P. marmoratus*, not very highly chitinised. *Ninth segment*: tergite moderately long, feebly chitinised especially posteriorly, dorsal surface with six

long, strong hairs, three on each side in an oblique row, posterior margin rounded, not notched, with the lateral finger-like processes reduced to small elevations each bearing a short hair; sternite deeply excavated in the middle line posteriorly. *Forceps*: side-pieces well developed, highly chitinised especially at the base; claspers long, poorly chitinised, of almost uniform width throughout, basal three-quarters clothed with minute hairs. *Harpes* highly chitinised; basal

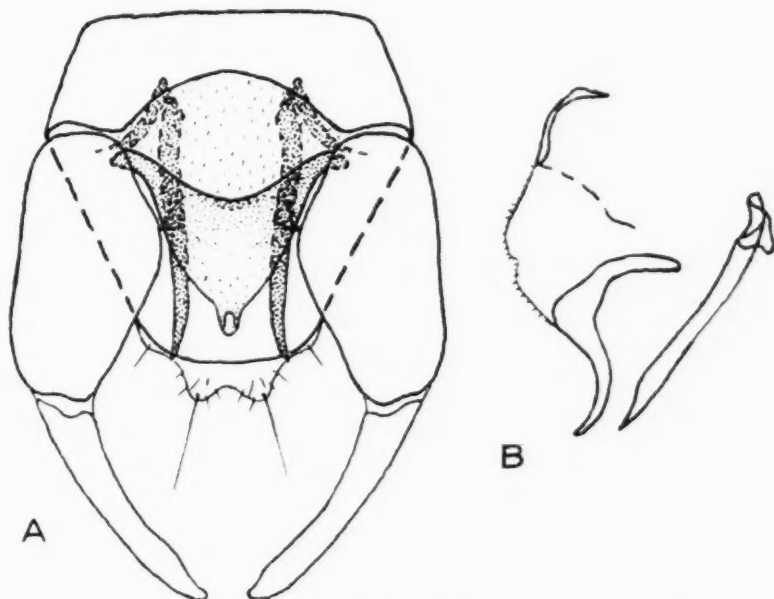


FIG. 4. *Thysanognathus* (*Prionognathus*) *melanostictus*, sp.n., male hypopygium. A—ventral view; B—lateral view of aedoeagus and harpe.  $\times$  c. 375.

portion directed laterally; distal portion a long, almost straight, rod-like structure directed posteriorly, reaching to the posterior margin of the ninth tergite, and tapering to a point. *Aedoeagus* highly chitinised basally, curved, shaped as shown in the figures. Membrane connecting the aedoeagus to the ninth sternite covered with spicules.

GOLD COAST: Accra, February, 1922; a single male, collected upon a window in the laboratory.

*Dasyhelea flavipicta*, sp. nov.

MEASUREMENTS.

Length of body (two females)	...	...	...	...	...	0.9 mm.
Length of wing	...	...	...	...	...	0.7 mm.
Greatest breadth of wing	...	...	...	...	...	0.26 mm.

*Head* brownish-yellow, the middle of the occiput brown. *Eyes* narrowly separated. *Clypeus* brownish-yellow. *Proboscis* and

palpi brownish-yellow; third segment of palp cylindrical, not inflated, nearly as long as the fourth and fifth segments together, sensory hairs very few. *Antennae*: torus dark brown, flagellum paler brown, with conspicuous short and long spines on all the segments, and rather short brown hairs. First segment small, hairless; segments four to ten oval to elongate-ovoid, slightly constricted at the apex but not flask-shaped, the length varying from one and three-fifths to one and four-fifths the width; segments eleven to fifteen slightly longer and more flask-shaped, length from about twice to two and a half times the width, the last segment not ending in a stylet but tapering to a blunt point. *Thorax* bright yellow with dark brown dorsal bands similar to those of *D. flava* but darker and not distinctly separated. Scutellum (fig. 5) bright yellow, very slightly darker at the sides, with two lateral and four centro-marginal bristles and a single central-sub-marginal, small hair.

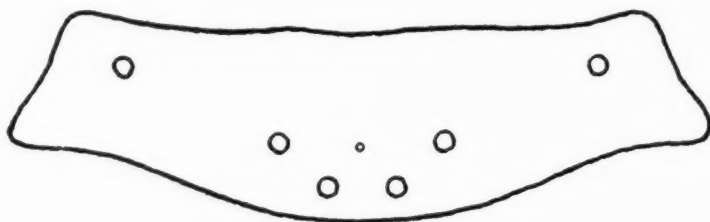


FIG. 5. *Dasybelea flavipicta*, sp.n., scutellum of female.  $\times$  c. 375.

Post-scutellum dark brown. Pleurae yellow or yellowish-brown. *Wings* clear, without spots, rather thickly clothed with long decumbent hairs which extend to the base between the fourth and fifth veins. The bifurcation of the fourth vein before the middle of the wing, that of the fifth vein at about the same level as the end of the costa. Halteres with bright yellow knobs and brownish stems. *Legs* almost uniformly light brown; claws short, equal, simple. *Abdomen*: dorsum dark brown, venter paler, with yellow pigment (soluble in caustic potash) visible laterally and, when the abdomen is distended, between the segments. Spermathecae similar to that of *D. flava*, single, highly chitinated, pyriform, length about  $45\mu$ ; the commencement of the duct is chitinated. Chitinous plates on the ventral aspect in the neighbourhood of the vulva unlike those of *D. flava*, and the tubular process present in that species apparently not developed (fig. 6).



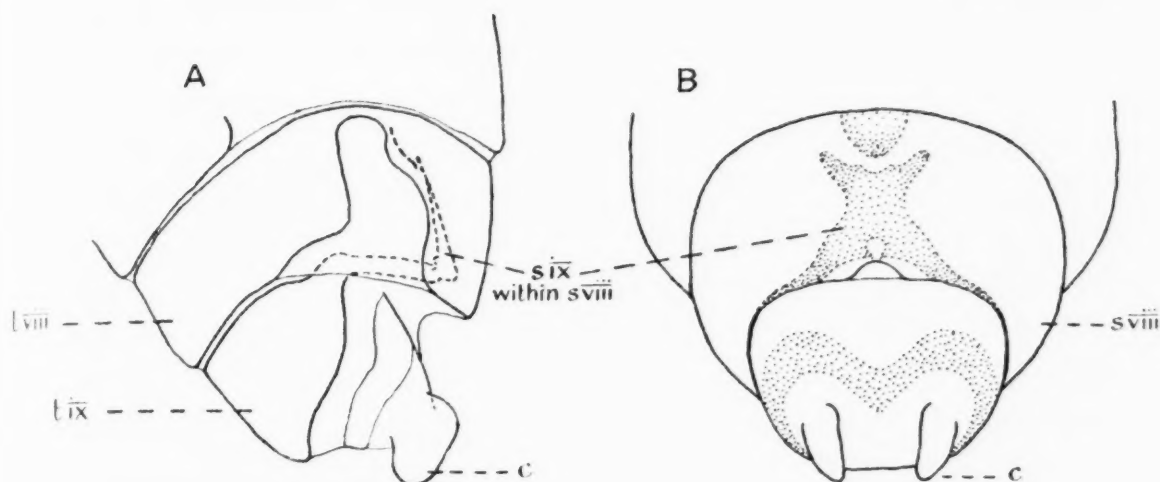


FIG. 6. *Dasyhelea flavipicta*, sp.n., posterior extremity of abdomen of female. A—lateral view; B—ventral view.  $\times$  c. 280. t—tergite; s—sternite; c—cerci.

GOLD COAST: Accra, 8th March, 1920; collected in the evening upon windows in the laboratory. This species closely resembles *D. flava*, but is darker, and differs from it in the form of the antennal segments as well as in other respects.

*Dasyhelea omoxantha*, sp. nov.

MEASUREMENTS.		Female.	Male.
Length of body (one specimen of each) ...	...	1.2 mm.	1.2 mm.
Length of wing ...	...	0.9 mm.	0.9 mm.
Greatest breadth of wing ...	...	0.3 mm.	0.3 mm.

*Head* dark brown. *Eyes* narrowly separated in both sexes. *Proboscis* dark brown. *Palpi* paler brown; fifth segment swollen at the end, fourth slightly shorter than the fifth, third slightly longer and only feebly inflated in its lower half. *Antennae* dark brown, bearing dark brown hairs and, on segments four to ten of the flagellum at least, short and longer, curved, spines; in the female, segments four to ten sub-spherical to ovoid, length from a little over once to once and two-thirds the width, segments eleven to fourteen rather more elongate, length from a little over once and two-thirds to nearly twice the width, the last segment broad, without a stylet; in the male, segments four to eleven spheroidal to ovoid, segments twelve to fourteen elongated, sub-equal, about three times as long as broad, binodose, the last segment slightly shorter, broader at the base and tapering to a conical end without a stylet. *Thorax* dark

brown with large, yellow, humeral patches. Scutellum almost entirely yellow, but slightly darker at the sides, bearing in both sexes two lateral and three centro-marginal bristles and no small hairs. Post-scutellum dark brown. Pleurae yellow above, dark brown beneath. *Wings* without spots. Decumbent hairs in the female fairly numerous and extending as a row almost to the base between the fourth and fifth veins, in the male fewer, not extending basally beyond the level of the cross-vein, and absent from the anal angle and the fork of the fifth vein. Costa not reaching as far as the middle of the wing in either sex; terminal cell well developed in the male but almost obsolete in the female. Fork of the fourth vein proximal to the middle of the wing in both sexes, that of the fifth vein in the female at about the level of the end of the costa, in the male slightly more distal. Halteres with pale yellow knobs. *Legs* brown, often a reddish colour, the proximal segments and the joints slightly darker; claws small, simple, equal, with a slight basal extension, and in the male with bifid tips. *Abdomen* dark brown, venter paler than dorsum. Spermatheca single, highly chitinised, pyriform, length  $32\mu$ , greatest breadth  $27\mu$ , the duct chitinised for only about  $2\mu$  or less at its commencement.

**HYPOPYGIUM** (fig. 7). *Ninth segment*: tergite broad, tapering only slightly, and scantily clothed with long, dark hairs, especially

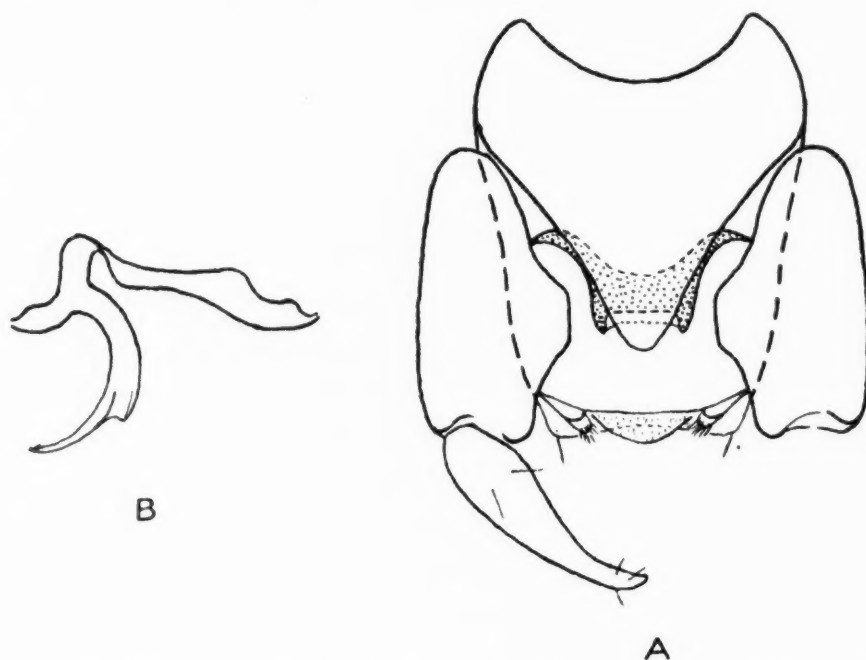


FIG. 7. *Dasybelea omoxantha*, sp.n., outlines of male hypopygium, ventral views. A—ninth segment, forceps, and aedeagus; B—harpes.  $\times c. 375$ .

in the middle line on the posterior quarter, posterior margin not notched, lateral angles squared, each with a single small hair and, more ventrally, a small process directed somewhat inwards and covered with short, stiff, hairs; sternite apparently prolonged posteriorly in the middle line as a delicate, more or less conical process. *Forceps*: side-pieces well developed, hairy; claspers single, rather highly chitinated; pubescent all over, and bearing in addition a few larger hairs at the base and apex. *Harpes*: basal portions very unequal and highly chitinated, appearance very variable in different specimens; from the right basal portion arises a long posterior projection which ends in a long pointed tip. *Aedoeagus* forming a broad, highly chitinated arch with a short posterior projection on each side.

NIGERIA (Southern Provinces): Calabar, February, 1922; one female and three males (Dr. E. C. Braithwaite). This species resembles in some respects both *D. luteoscutellata* and *D. inconspicua*, especially the former, but may be distinguished by the colour of the halteres and by the large, yellow, humeral patches. The hypopygium of the male is characteristic.

*Atrichopogon africanum*, Ingram and Macfie

MEASUREMENTS.

Length of body (one male)	...	...	...	...	...	1.7 mm.
Length of wing	...	...	...	...	...	1.3 mm.
Greatest breadth of wing	...	...	...	...	...	0.4 mm.

*Head* dark brown, clothed with dark brown hairs. Eyes pubescent, rather sparsely and apparently only on the upper halves; contiguous but with the facets narrowly separated. Clypeus, palpi, and proboscis dark brown. First palpal segment small, second about as long as the fifth, third longer, somewhat inflated, and bearing a well developed sensory cup at about its middle, fourth about half the length of the third, and fifth rather longer than the fourth and only slightly expanded at its end. *Antennae*: first segment and torus dark brown, the former a mere ring of chitin, the latter large, very dark, hairless. Flagellum paler brown, the terminal segments somewhat darker than the rest, with a well developed plume of brownish hairs. The twelfth segment is slightly prolonged at its distal end, length rather more than four times the

breadth, the last three segments elongated, about seven times as long as broad, the fourteenth being the shortest and the fifteenth the longest and ending in a long (about  $20\mu$ ), pointed, stylet (fig. 8 *a*). The combined lengths of segments twelve to fifteen is rather greater than the combined lengths of segments four to eleven, namely (excluding the stylet, which measures about five units) 125 to 111, or 1.12 to 1. *Thorax* uniformly dark brown. *Scutellum* dark brown, bearing two admedian and two lateral bristles and

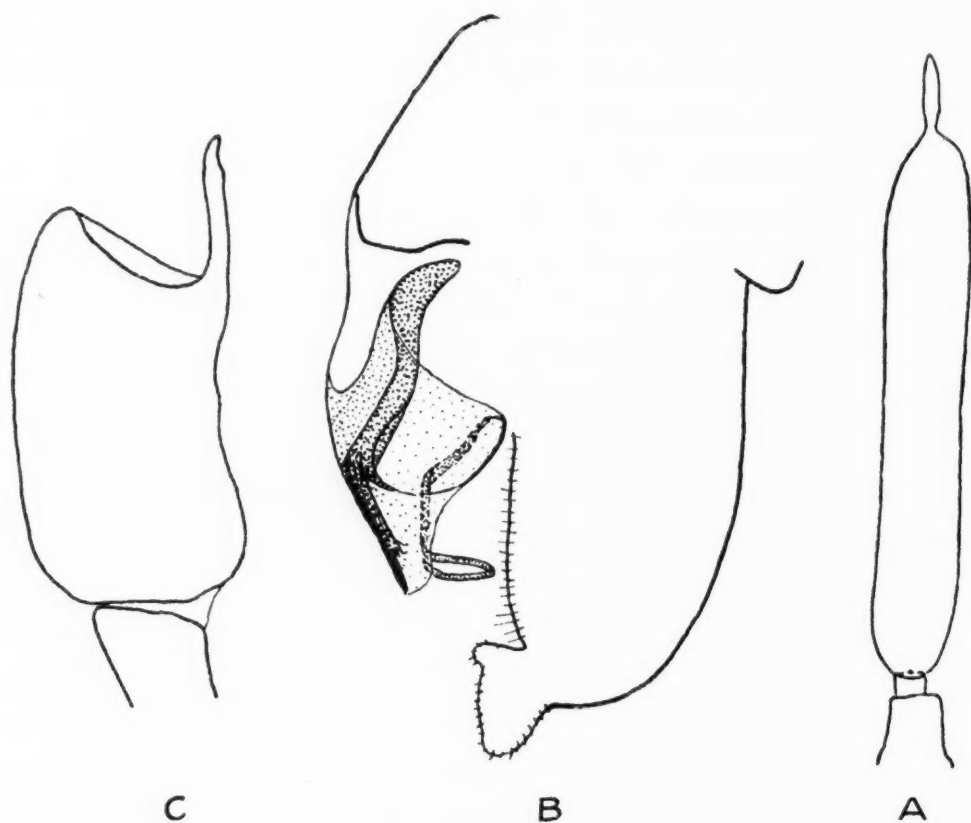


FIG. 8. *Atrichopogon africanum*, I. & M., *A*—outline of the last segment of the antenna of the male; *B*—hypopygium of male, aedeagus in lateral view; *C*—side-piece to show the dorsal root-like process. All  $\times c. 375$ .

about six small hairs. Post-scutellum dark brown. Pleurae dark brown. *Wings* clear, unspotted; surface covered by microtrichia but without longer decumbent hairs. Venation as in *A. (K.) ochrosoma*. Halteres with rather dark brown knobs. *Legs* almost uniformly brown, tarsal segments rather darker than the others, unarmed. Claws equal, small, about half the length of the fifth tarsal segment, bifid at the tips. Empodium well developed, hairy,

at least as long as the claws. *Abdomen* dark brown, but not so dark as the thorax.

**HYPOPYGIUM** (fig. 8, B and C, and fig. 9). Generally similar to that of *A. (K.) ochrosoma*. *Ninth segment* well chitinised: tergite long, bearing (especially on its posterior fourth) a number of strong hairs, posterior margin rounded, without lateral, finger-like processes; sternite deeply notched, bearing a few hairs. *Forceps* highly chitinised, normal in form; side-pieces with large, curved, dorsal root-like processes which articulate with the proximal ends of the aedoeagus; claspers rather strongly chitinised, entirely covered

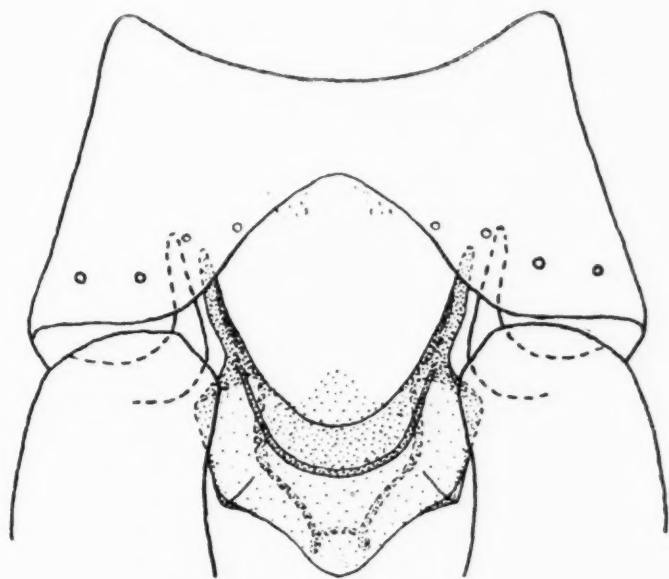


FIG. 9. *Atrichopogon africanum*, I. & M., part of male hypopygium, ventral view, showing the ninth sternite and the aedoeagus.  $\times$  c. 375.

by pubescent hairs, and bearing in addition a few longer hairs. *Harpes* apparently wanting. *Aedoeagus* similar to that of *A. (K.) ochrosoma*, its form is somewhat variable, apparently depending on the degree to which it is protruded.

**GOLD COAST:** Accra; taken in the evening upon the windows of the laboratory. This insect resembles *Atrichopogon africanum*, I. and M., of which only the female is known, and we have, therefore, described it as the male of that species. It should be made clear, however, that this association is not without doubt, and may subsequently require correction.



*Atrichopogon chrysospherotum*, I. and M.

## MEASUREMENTS.

Length of body (one male)	...	...	...	...	...	1.1 mm.
Length of wing	...	...	...	...	...	0.9 mm.
Greatest breadth of wing	...	...	...	...	...	0.26 mm.

This male insect resembles the female of *A. chrysospherotum* in most respects; the following characters, including the points of difference, may, however, be noted.

*Head*: eyes very sparsely hairy as in the female, the pubescence being most distinct at the sides near the anterior margin; contiguous above but with the facets narrowly separated. First palpal segment small, second and third sub-equal and rather small, the third only slightly inflated and with quite a minute sensory pit, fourth slightly shorter and broader than the third, fifth still smaller, with a rounded and undilated end (see fig. 10 C). *Antennae*: first segment a mere

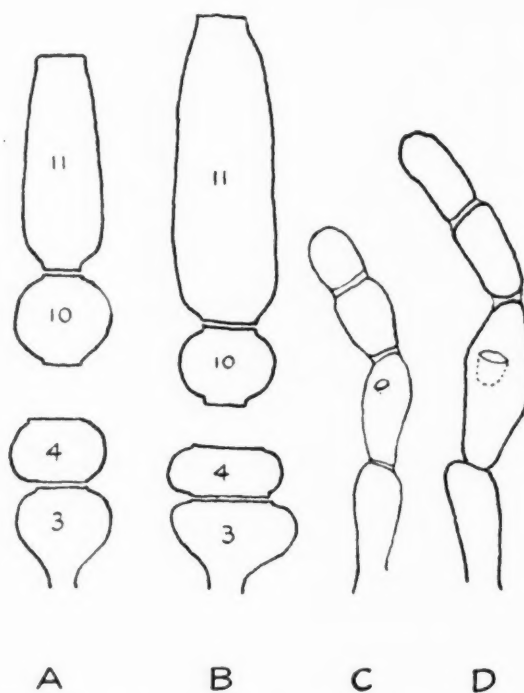


FIG. 10. Third and fourth and tenth and eleventh segments of the antenna of the female: A—*Atrichopogon chrysospherotum*; B—*Atrichopogon bomoiium*. ( $\times 375$ .) Last four segments of the palp of the male; C—*A. chrysospherotum*; D—*A. bomoiium*. ( $\times 375$ .)

ring of chitin without hairs; torus large, dark brown, bearing one or two hairs; flagellum darkish brown, the last three segments darker than the rest. The twelfth segment is only slightly produced distally, length about three times the breadth; the last three segments

elongated, sub-equal, about five times as long as broad, the fifteenth somewhat wider than the other two and ending in a long stylet of the usual form. The combined lengths of segments twelve to fifteen only slightly greater than the combined lengths of segments four to eleven, namely (excluding the stylet which measures about four units or  $15\mu$ ), 77 units to 75, or 1.02 to 1. *Thorax*: scutellum without small hairs. *Wings* covered by microtrichia, but without longer, decumbent hairs. Halteres with yellow knobs, rather paler than in the female. *Legs* as in the female; claws equal, small, about half the length of the fifth tarsal segment, simple, bifid at the tips. Empodium hairy, large, as long as the claws. *Abdomen* darkish brown, containing a substance of a yellow colour which is soluble in caustic potash but not in carbolic acid.

**HYPOPYGIUM** (figs. 11, A and B). Highly chitinised, closely resembling that of *A. homoiium*, the main point of distinction being

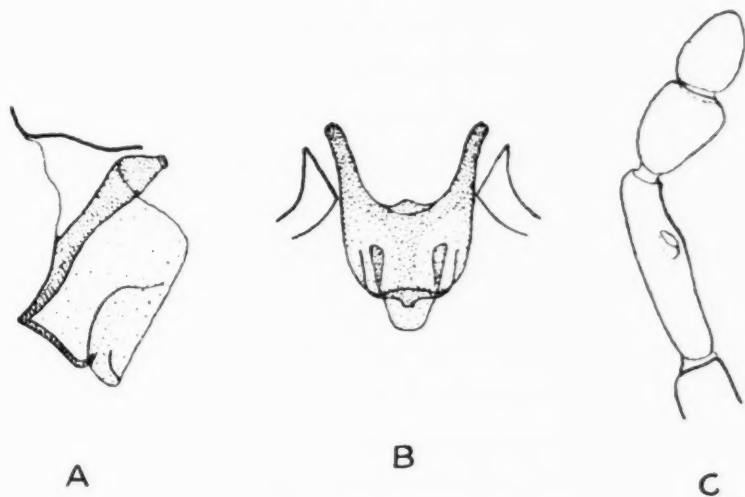


FIG. 11. *Atrichopogon chrysosphaerotum*, I. & M., aedeagus. A—lateral view; B—ventral view. *Atrichopogon acanthocolpum*, sp.n. C—last three segments of palp of female. All  $\times c. 375$ .

the aedeagus, the form of which in ventral and lateral views is shown in the figures.

**GOLD COAST**: Accra, 1921; taken in the evening upon a window in the laboratory. This insect resembles *A. chrysosphaerotum*, and is, therefore, described here as the male of that species. It should, however, be clearly understood that this association is merely conjectural, and may or may not be confirmed by further experience.

*Atrichopogon homoius*, I. and M.

An examination of further specimens of this species enables us to add one or two points to our previous description of the female.

*Head*: eyes, as in *A. chrysospherotum*, sparsely hairy, the pubescence being most distinct laterally near the anterior margin; contiguous but with the facets narrowly separated. Third palpal segment (fig. 10 D) rather longer than in *A. chrysospherotum*, and about once and one-third the length of either the second or fourth segments. *Antennae*: as in *A. chrysospherotum*, the first segment is large and bears several hairs, and the torus is roughly spherical and also bears a few hairs. In both *A. chrysospherotum* and *A. homoius* segments four to ten are broad and short (fig. 10, A and B), but in the latter the basal ones are rather broader, the fourth segment, for example, measuring in one instance 4 by 9 units, as compared with 4 by 7 in the former. The last five segments range in length from about two and a half to nearly three times the breadth in *A. chrysospherotum*, and from about three to over five times the breadth in *A. homoius*. The basal segments of the flagellum in *A. homoius* are shorter and broader, and the apical segments are more elongated than in *A. chrysospherotum*, the ratio of the combined lengths of segments four to ten to the combined lengths of segments eleven to fifteen being in one instance 34 to 115 units (1 to 3.4) in the former, and 36 to 91 units (1 to 2.5) in the latter. In these measurements the stylet (which measures about three units in the former and four in the latter) is not included. *Wings*: the decumbent hairs near the tip of the wing are rather variable, in one of our specimens there were eight on one wing and twelve on the other; there may be no decumbent hairs between the rami of the fourth vein.

We are also now able to give a description of the male.

## MEASUREMENTS.

Length of body (one male)	...	...	...	...	...	1.3 mm.
Length of wing	...	...	...	...	...	1.0 mm.
Greatest breadth of wing	...	...	...	...	...	0.3 mm.

The male is in most respects similar to the female, but the following points may be noted. *Head*: eyes as in the female. Palpi as in the female. *Antennae*: first segment a mere ring of

chitin, without hairs; torus large, dark brown, bearing one or two hairs; flagellum pale brown basally, the last three segments dark brown. The twelfth segment is only slightly elongated, length about three times the breadth; the last three segments are more elongated, sub-equal, about six times as long as broad, the fifteenth segment ending in a long stylet. The combined length of segments twelve to fifteen considerably greater than that of segments four to eleven, namely (excluding the stylet which measures about four units, or  $15\mu$ ), 107 units to 77, or 1.4 to 1. *Thorax*: scutellum bearing, in addition to the setae, apparently only two small hairs, one on each side. *Wings* longer and narrower than in the female, and without decumbent hairs. Halteres with yellow knobs, rather paler than in the female. *Legs* as in the female: first tarsal segments of the hind legs not quite three times the length of the second. Claws small, equal, with bifid ends.

**HYPOPYGIUM** (fig. 12 A and B). *Ninth segment* well chitinised: tergite long, sparsely clothed with long, dark-brown hairs which

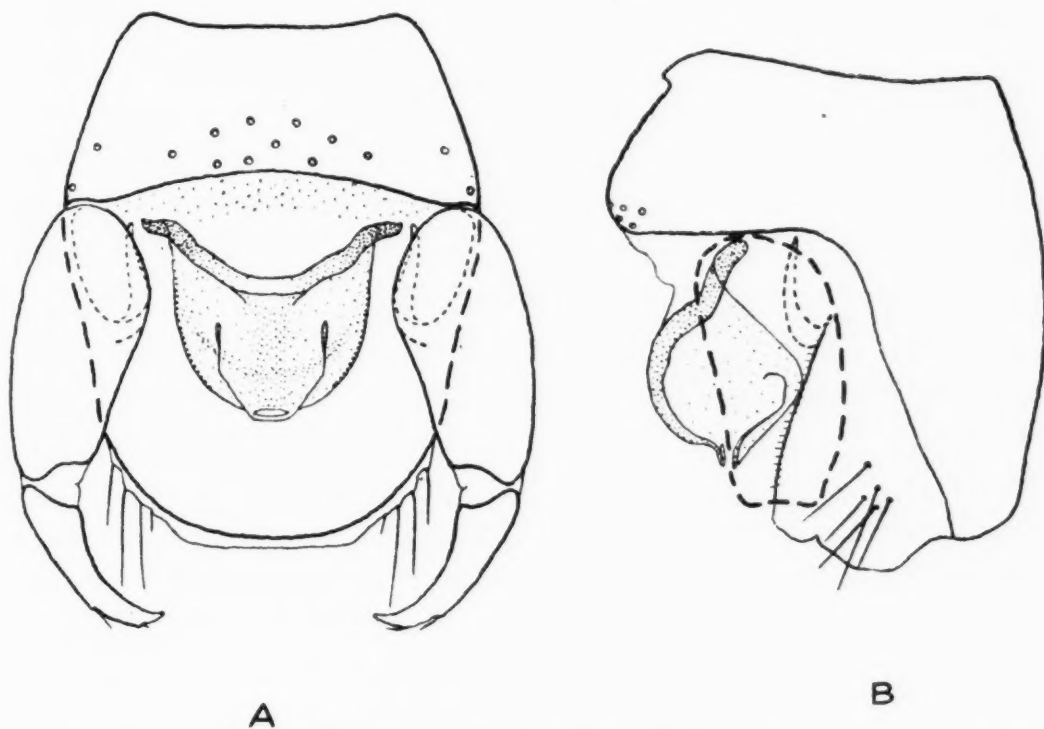


FIG. 12. *Atrichopogon homoius*, I. & M., outlines of male hypopygium. A—ventral view; B—lateral view.  $\times$  c. 375.

are most numerous on the posterior fourth, posterior margin rounded, without a notch and without lateral finger-like processes; sternite hardly at all excavated in the middle line posteriorly, but bearing a

group of about ten stout hairs in this position. Membrane connecting the ninth sternite with the aedoeagus studded with small spicules on its anterior half. *Forceps* normal, not very highly chitinated. *Harpes* apparently absent. *Aedoeagus* of characteristic form (see fig. 12 A and B), proximal arch low, wide, feebly chitinated in the middle.

GOLD COAST: Accra, 10th December, 1921; two females, taken in the evening upon a window in the laboratory. Aburi, 3rd December, 1921; one male and one female, reared from material from a dead tree.

*Atrichopogon acanthocolpum*, sp. nov.

MEASUREMENTS.

Length of body (one female)	...	...	...	...	...	1.0 mm.
Length of wing	...	...	...	...	...	0.9 mm.
Greatest breadth of wing	...	...	...	...	...	0.37 mm.

*Head* very dark brown. Eyes densely hairy all over; contiguous above but with the facets rather widely separated. Clypeus, proboscis, and palpi dark brown. First palpal segment relatively long, second and fourth sub-equal, the former being slightly the longer and the latter being unusually broad, third segment about twice the length of the fourth, narrow, only very slightly inflated, and bearing a small sensory pit, fifth shorter than the fourth, narrowed at its distal end, not dilated (fig. 11 C). *Antennae* very dark and bearing short, dark brown hairs: first segment well developed, bearing a few hairs; torus very dark brown, bearing a few hairs; flagellum dark brown, segments four to ten sub-equal, sub-spherical, segments eleven to fifteen elongated, sub-equal, about three times as long as broad, the eleventh segment being slightly the shortest, the fifteenth slightly the longest and broadest and terminating in a long stylet. The combined lengths of segments eleven to fifteen (excluding the stylet) over twice the combined lengths of segments four to ten, namely, 93 units to 43, or 2.1 to 1. *Thorax* very dark brown, with a narrow, paler, sub-lateral line on each side, which expands posteriorly just before the scutellum into a pale patch. Scutellum dark brown, bearing two admedian bristles and two small hairs in place of the lateral bristles. Post-scutellum and pleurae dark brown. *Wings* clear, unspotted, covered by micro-



trichia but without longer, decumbent, hairs. Venation similar to that of *A. africanum*, but both radial cells narrow, slit-like, almost obsolete. Halteres almost colourless in the specimen when examined, that is, after preservation in alcohol. *Legs* yellowish-brown, tarsal segments somewhat darker. Femora and tibia rather sparsely clothed with hairs and with unarmed shafts. First tarsal segment of the hind legs about two and a half times the length of the second. Fourth tarsal segment bell-shaped. Claws equal, small, about half the length of the fifth tarsal segment, with a small notch about the middle. Empodium hairy, as long as the claws. *Abdomen* darkish brown, venter paler than the dorsum: in the neighbourhood of the genital opening is an armature of stout spines, namely, a single stout median spine terminating in several small sharp points on the posterior margin of the seventh sternite, a transverse, comb-like, row of stout, relatively blunt, spines on the eighth sternite, composed of about seven spines on each side, and just posterior to this row, two lateral patches, each of about a dozen similar but rather smaller spines. Spermatheca single, highly chitinated, pyriform, with 'pale spots' at the base; length about  $85\mu$ , greatest breadth about  $60\mu$ , the commencement of the duct chitinated for a short distance, about  $8\mu$ .

NIGERIA, Southern Provinces: Calabar, February, 1922 (Dr. E. C. Braithwaite).

*Atrichopogon kelainosoma*, sp. nov.

MEASUREMENTS.

Length of body (one male)	...	...	...	...	...	1.8 mm.
Length of wing	...	...	...	...	...	1.3 mm.
Greatest breadth of wing	...	...	...	...	...	0.4 mm.

*Head* dark brown, clothed with dark brown hairs. Eyes hairy, the pubescence, however, almost restricted to the lateral portions of the anterior borders; contiguous, but with the facets narrowly separated. Clypeus, palpi, and proboscis dark brown. First palpal segment small but distinct, second and fourth about the same size, third longer, about once and a half the length of the fourth, slightly inflated in the middle and furnished with a deep sensory pit, fifth slightly longer than the fourth and only slightly dilated at its end. *Antennae* rather dark brown with large plumes of brown

hairs: first segment a mere ring of chitin; torus large, dark brown, bearing one or two hairs; flagellum paler brown and almost unicolourous, the twelfth segment somewhat produced distally, length about four times the breadth, the last three segments elongated, about seven to eight times as long as broad, the fourteenth being the shortest, and the fifteenth slightly the longest and ending in a long stylet. The combined lengths of segments twelve to fifteen is considerably greater than that of segments four to eleven, namely (excluding the stylet, which is about five units long), 131 units to 96, or 1.36 to 1. *Thorax* uniformly dark brown. Scutellum dark brown, bearing two admedian and two lateral bristles and four small hairs. Post-scutellum dark brown. Pleurae dark brown. *Wings* clear, unspotted, without decumbent hairs. Venation as in *A. africanum*. Halteres with darkish brown knobs. *Legs* almost uniformly brown, tarsal segments, however, rather darker than the rest, unarmed. Claws equal, small, about half the length of the fifth tarsal segment, with a slight indication of a notch, and with bifid tips. Empodium well developed, hairy; at least as long as the claws. *Abdomen* dark brown, but not so dark as the thorax.

**HYPOPYGIUM** (fig. 13). Similar to that of *Atrichopogon africanum*. *Ninth segment* well chitinised: tergite as in

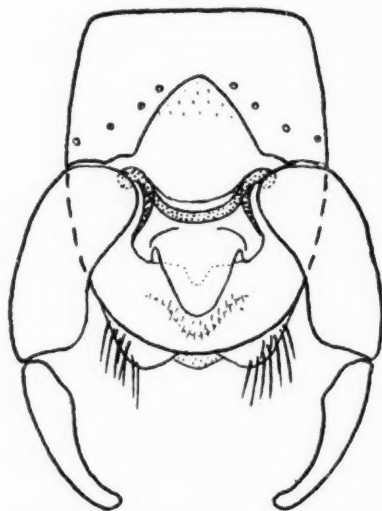


FIG. 13. *Atrichopogon kelainosoma*, sp.n., outline of male hypopygium, ventral view.  $\times$  c. 185.

*A. africanum*; sternite deeply excavated in the middle line posteriorly as in *A. africanum*, and bearing similar hairs, but with the small

spicules extending right across the apex of the notch. *Forceps* highly chitinised, similar to those of *A. africanum*. Harpes apparently absent. *Aedoeagus* similar to that of *A. africanum* but differing in detail as shown in the figures.

GOLD COAST: Accra; taken in the evening upon a window in the laboratory. This species closely resembles *A. africanum*, but may be distinguished by the following amongst other characters: the lesser degree of hairiness of the eyes, the greater length of the fourth palpal segment, and the form of the aedoeagus.

*Atrichopogon acosmetum*, sp. nov.

MEASUREMENTS.

Length of body (two males)	...	...	...	...	...	1.5 mm.
Length of wing	...	...	...	...	...	1.2 mm.
Greatest breadth of wing	...	...	...	...	...	0.3 mm.

*Head* dark brown, clothed with dark brown hairs. Eyes pubescent, as in *A. kelainosoma*, especially at the sides near the anterior margin; contiguous, but with the facets narrowly separated. Clypeus, palpi, and proboscis dark brown. First palpal segment small, second and fourth sub-equal, fifth about the same size or a little shorter and not expanded at its end, third about one and a half times the length of the fourth, inflated in the middle, and furnished with a well-developed sensory pit. *Antennae* rather dark, with plumes of darkish-brown hairs; first segment a mere ring of chitin; torus large, dark brown; flagellum segments paler brown, excepting the last four which are darkish brown. The twelfth segment is somewhat produced distally, length rather more than three times the breadth; the last three segments are elongated, sub-equal, about six times as long as broad, the fifteenth segment being slightly the longest and ending in a long stylet. The combined lengths of segments twelve to fifteen slightly greater than that of segments four to eleven, namely (excluding the stylet which measures about five units, or about  $20\mu$ ), 107 to 90 units, or 1.18 to 1. *Thorax* dark brown, with a small pale spot on each side immediately in front of the scutellum near its lateral margins, and with two conspicuous long hairs, one on each side, a little in front of them. Scutellum dark brown, bearing two lateral and two admedian bristles and one or two small hairs. Post-scutellum dark brown.

Pleurae dark brown. *Wings* clear, unspotted; surface covered by microtrichia, but without longer decumbent hairs. Venation as in *A. africanum*, first radial cell small and slit-like. Halteres with cream-coloured or whiteish, slightly infuscated, knobs. *Legs* brown or yellowish-brown, tarsal segments darker than the others. Claws small, about half the length of the fifth tarsal segment, equal, with a small barb, and with bifid tips. Empodium well developed, hairy, as long as the claws. *Abdomen* darkish brown.

**HYPOPYGIUM** (fig. 14). Closely resembling that of *A. africanum*. *Ninth segment* well chitinised: tergite long, sparsely clothed with long, dark hairs, posterior margin rounded, without a notch and

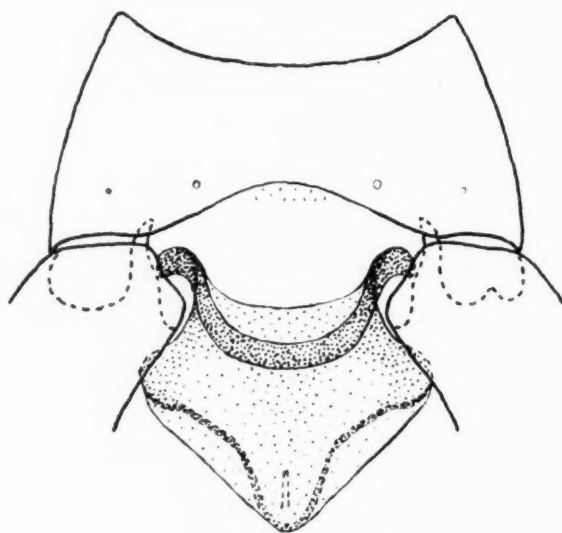


FIG. 14. *Atrichopogon acosmetum*, sp.n., part of male hypopygium, ventral view, showing ninth sternite and aedeagus.  $\times$  c. 375.

without lateral finger-like processes; sternite notched in the middle line posteriorly, but not so deeply as in *A. africanum*, bearing one or two hairs on each side of the notch. Membrane joining the ninth sternite to the aedeagus studded with spicules on that part which occupies the apex of the notch. *Forceps* highly chitinised, normal in form; dorsal root-like processes of the side-pieces shorter than in *A. africanum*. *Harpes* apparently wanting. *Aedeagus* similar to that of *A. kelainosoma*.

**GOLD COAST:** Accra, 24th December, 1921, and upon an unknown date in 1920; two males taken in the evening upon windows of the laboratory. This species closely resembles *A. kelainosoma*, but is smaller, and may be distinguished from it by the colour of the

halteres and the details of the hypopygium as well as by other characters.

We also collected in the Accra laboratory a single specimen of a midge which was probably the female of this species. The specimen is unfortunately imperfect. The following is a brief description of it:—

#### MEASUREMENTS.

Length of body (one female)	...	...	...	...	...	1.4 mm.
Length of wing	...	...	...	...	...	1.0 mm.
Greatest breadth of wing	...	...	...	...	...	0.4 mm.

*Head* dark brown, clothed with dark brown hairs. Eyes very sparsely hairy, the pubescence being most distinct at the sides near the anterior margin; contiguous above but with the facets narrowly separated. Clypeus, palpi and proboscis brown. First palpal segment small, second rather longer than the fourth, third about once and a half the length of the fourth, somewhat inflated, with a large sensory pit, fifth shorter than the fourth, with a rounded end which is not dilated. *Antennae*: first segment large, bearing a few hairs; torus dark brown, more or less rounded, bearing a few hairs; flagellum unfortunately missing. *Thorax* dark brown. Scutellum dark brown, bearing two lateral and two admedian bristles, and four small hairs (two on each side). Post-scutellum dark brown. Pleurae dark brown. Wings clear, unspotted, covered by microtrichia; three or four longer decumbent hairs near the tip of the wing and about a dozen along the upper ramus of the fourth vein on its distal portion, but no decumbent hairs on the other parts of the wing surface. Venation as in *A. africanum*. Halteres with white knobs which have a pale-yellowish tint. *Legs* almost uniformly yellowish-brown; first tarsal segment of the hind legs nearly three times as long as the second; claws equal, small, about half the length of the fifth tarsal segment, with a small barb. Empodium hairy, long, at least as long as the claws. *Abdomen* dark brown, venter rather paler than dorsum. Spermatheca single, highly chitinated, oval, measuring about  $46\mu$  by  $42\mu$ , the duct chitinated for only a very short distance (about  $2\mu$ ) at its commencement.

**GOLD COAST:** Accra, 1921; a single female collected in the evening upon a window in the laboratory.



*Atrichopogon hesperium*, sp. nov.

## MEASUREMENTS.

Length of body (one male)	...	...	...	...	...	1.4 mm.
Length of wing	...	...	...	...	...	1.1 mm.
Greatest breadth of wing	...	...	...	...	...	0.3 mm.

*Head* dark brown. Eyes sparsely hairy, the pubescence, as in *A. kelainosoma*, most distinct at the sides near the anterior margin; contiguous but with the facets narrowly separated. Clypeus, palpi, and proboscis brown. Second and fourth palpal segments subequal, third small, only slightly longer than the fourth (twelve to nine units), slightly inflated, and with a small sensory pit. *Antennae*: first segment a mere ring of chitin; torus large, dark brown, bearing about six hairs; flagella unfortunately missing. *Thorax* dark brown. Scutellum darkish brown, bearing two lateral and two admedian bristles and no small hairs. Post-scutellum dark brown. Pleurae brown. *Wings* clear, unspotted, without decumbent hairs; venation as in *A. africanum*. Halteres with white knobs. *Legs* almost uniformly brown; claws unfortunately missing. *Abdomen* darkish brown.

**HYPOPYGIUM** (fig. 15). *Ninth segment* well chitinised: tergite moderately long, sparsely clothed with hairs, posterior margin rounded, without lateral finger-like processes; sternite not notched,

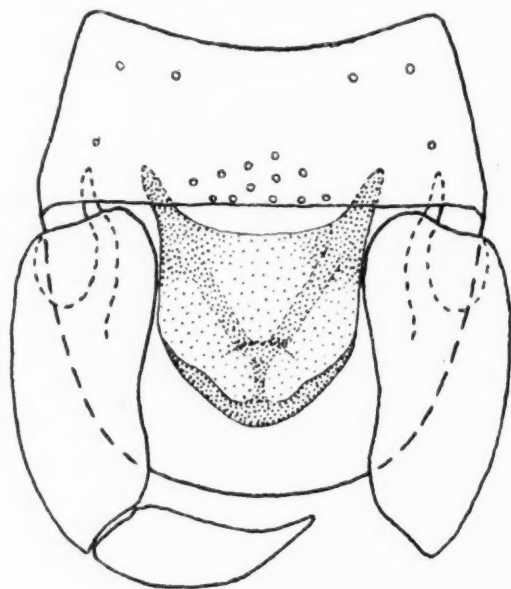


FIG. 15. *Atrichopogon hesperium*, sp.n., outline of male hypopygium, ventral view.  $\times$  c. 375.

but with a group of a dozen stout setae in the middle line posteriorly. *Forceps* normal, dorsal root-like processes of the side-pieces long, claspers short, with broad, hairy bases and strong, sharply pointed, almost claw-like extremities. *Aedoeagus* rather highly chitinated and characteristic in form (see figure).

GOLD COAST: Accra, 24th December, 1921; collected in the evening upon a window in the laboratory. This species is described here, although at present we possess only a single, damaged specimen, on account of the characteristic form of the hypopygium.

*Stilobezzia limnophila*, sp. nov.

MEASUREMENTS.

Length of body (one male)	...	...	...	...	...	0.9 mm.
Length of wing	...	...	...	...	...	0.8 mm.
Greatest breadth of wing	...	...	...	...	...	0.23 mm.

A small, delicate, greenish-brown midge. *Head* dark brown. Eyes bare, contiguous above but with the facets rather widely separated. Clypeus, proboscis, and palpi brown. Second and third palpal segments sub-equal, third not inflated and without a sensory pit, but with a small anterior depression from which arise a few sensory hairs, fourth slightly shorter than the third, cylindrical, fifth longer than any of the other segments, widely dilated at its end, pyriform. *Antennae*: first segment moderately large, brown; torus large, rounded, yellowish-brown, bearing a few hairs; flagellum unfortunately missing. *Thorax* almost uniformly dark greenish-brown. *Pleurae* dark greenish-brown. *Scutellum* dark greenish-brown, bearing two admedian and two (smaller) lateral bristles, but no small hairs. *Post-scutellum* dark greenish-brown. *Wings* clear, without dark markings; surface covered by microtrichia but without longer, decumbent hairs. Venation as in *S. spirogyrae*. *Halteres* with dark, greenish-brown knobs. *Legs* long, almost colourless, but the knees and the apices of the tibiae are slightly yellowish-brown. Rows of small spines on the first and second tarsal segments as in *S. spirogyrae*; apical pairs of spines on the tarsal segments poorly developed, and other spines apparently absent. Third tarsal segment on all the legs bell-shaped, fourth cordiform. First tarsal segment of hind legs about twice the length of the second. Claws equal, small, less than half the length of the fifth tarsal

segment, bifid at the tips. Empodium rudimentary. *Abdomen* dark greenish-brown dorsally, excepting the first two segments which are pale; venter paler.

**HYPOPYGIUM** (fig. 16). *Ninth segment*: tergite sparsely clothed with long hairs, rather short, tapering slightly, the posterior margin rounded, notched, without lateral finger-like processes; sternite very

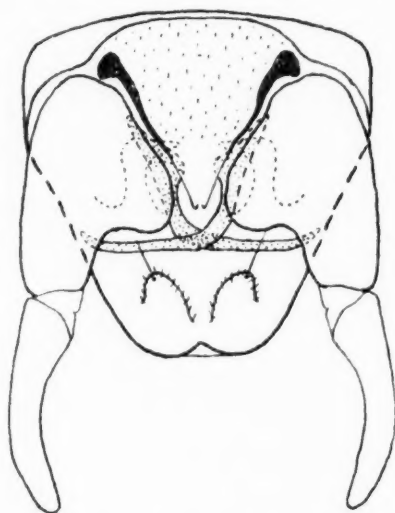


FIG. 16. *Stilobezzia limnopila*, sp.n., hypopygium of male, ventral view.  $\times c. 375$ .

short, slightly excavated. *Forceps* feebly chitinised: side-pieces sparsely clothed with long hairs, each with a broad, inwardly-projecting, basal process, and a highly chitinised, beak-like, dorsal root-like process; claspers blunt, clothed with minute hairs and a few rather longer, delicate hairs. *Harpes* long, slender, highly chitinised, crossing in the middle line; basal portion foot-like, distal portion long, tapering gradually. *Aedoeagus* V-shaped, the membrane joining it to the ninth sternite spiculated.

**GOLD COAST**: Accra, 26th December, 1921; reared from mud from the margin of a pool near the station for the Weshiang Railway.

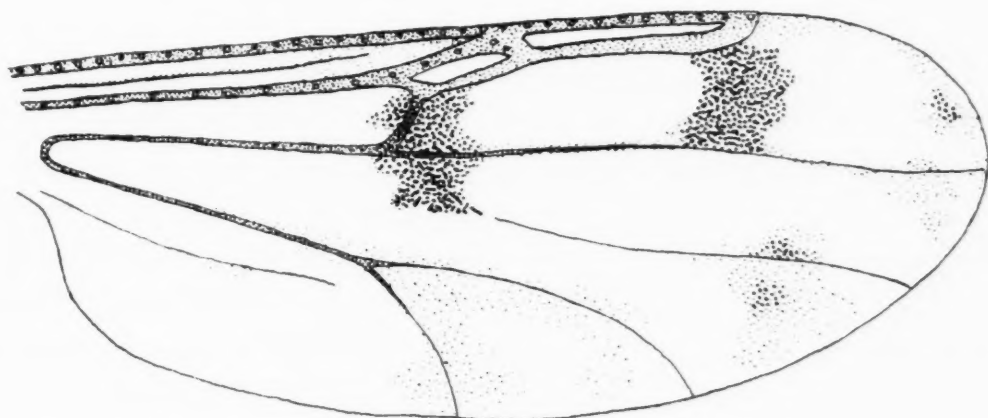
*Monohalea nigeriae*, sp. nov.

MEASUREMENTS.

Length of body (one female)	...	...	...	...	...	1.2 mm.
Length of wing	...	...	...	...	...	0.8 mm.
Greatest breadth of wing	...	...	...	...	...	0.3 mm.

This insect is generally similar to *Monohalea litoraurea*. *Head* dark brown. Eyes above widely separated posteriorly and just

touching anteriorly. Clypeus, proboscis, and palpi dark brown. Stylets of the proboscis highly chitinated, mandibles strongly serrated at their ends. First palpal segment very small, second and fourth sub-equal, third about once and a half the length of the fourth, inflated about its middle, bearing a large sensory pit, fifth slightly longer than the fourth, somewhat dilated at its end. *Antennae* brown: first segment rather large, darkish brown, bearing a few hairs; torus rounded, rather dark yellowish-brown, bearing a few hairs; flagellum paler brown, the distal halves of the basal segments and the whole of the last five segments darker. Segments four to ten almost sub-equal, about twice as long as broad or a little longer; segments eleven to fourteen elongated, about four times as long as broad, the last segment slightly longer (24 units by 5), tapering at its extremity. Combined lengths of segments eleven to fifteen rather greater than the combined lengths of segments four to ten, namely, about 106 units to 93, or 1.14 to 1. *Thorax* darkish brown. Scutellum darkish brown, darker in the centre and at the sides, bearing two admedian and two lateral bristles, and six short hairs. Post-scutellum dark brown. Pleurae dark brown. *Wings* (fig. 17)



the segments and armatures of spines as in *M. litoraurea*; fourth tarsal segments cylindrical. First tarsal segment of the hind legs two and a half times as long as the second, and with a double bend at the base. Claws on the fore and middle legs equal, rather long, about three-fifths the length of the fifth tarsal segment, each with a small basal tooth; on the hind legs single, longer (but not so long as in *M. litoraurea*), about as long as the fifth tarsal segment, with a basal tooth. Empodium rudimentary. *Abdomen* grey-brown with dark markings in the fresh state, almost uniformly dark brown after preservation; tip of the body pale, almost white. Spermathecae two, highly chitinated, oval or sub-spherical, unequal, diameters in the single specimen examined approximately  $57\mu$  by  $45\mu$ , and  $50\mu$  by  $50\mu$ ; the commencement of the duct chitinated for only a very short distance.

NIGERIA, Southern Provinces: Lagos, 26th November, 1921 (Dr. H. Andrew Foy); collected in the evening upon a lamp-shade.

*Eukraiohelea foyi*, sp. nov.

MEASUREMENTS.

Length of body (one female)	...	...	...	...	...	1.7 mm.
Length of wing	...	...	...	...	...	1.5 mm.
Greatest breadth of wing	...	...	...	...	...	0.5 mm.

This insect is grey, with brown markings, and in many respects resembles *Eukraiohelea africana*.

*Head* dark brown. Eyes bare, narrowly separated above. Clypeus, proboscis and palpi dark brown. Stylets of the proboscis very highly chitinated. First palpal segment very small, second and fourth sub-equal, cylindrical, third and fifth sub-equal, slightly longer than the fourth, the third only very slightly inflated and bearing a small sensory pit near its distal end, the fifth slightly longer and slightly dilated at its end. *Antennae* as in *E. africana*; combined lengths of segments eleven to fifteen (excluding the stylet which measures about four units, or  $15\mu$ ) more than twice the combined lengths of segments four to ten, namely, about 253 units to 121, or 2.1 to 1. *Thorax* brown, darkest anteriorly. Scutellum brown, bearing two admedian and two lateral bristles and one small hair on each side. Post-scutellum dark brown. Pleurae grey: above the coxae of the fore and middle legs is a brown spot. *Wings*



clear, unspotted, covered by microtrichia but without decumbent hairs. Venation similar to that of *E. africana*, but the terminal part of the first vein and the anterior cross vein form an almost straight, though oblique, line, and the fork of the fourth vein is slightly distal to that of the fifth (fig. 18). Halteres with white knobs bearing a few small hairs; stalks brownish, bases of the knobs rather deeply infuscated. Legs pale brown, almost colourless, with dark brown knee-spots, the infuscation on the hind legs extending below the knee about one-third of the length of the tibiae, and dark brown apical spots on the fore and hind tibiae. Armature of spines and form of the segments as in *E. africana*. Claws as in *E. africana*. Empodium absent. Abdomen grey with brown dorsal markings, namely, on each side of segments two to six a broad L-shaped mark,



FIG. 18. *Eukraiohelea foyi*, sp.n., part of wing of female to show venation.  $\times$  c. 90.

the vertical limbs being lateral and the horizontals reaching transversely towards the middle line but falling somewhat short of it. Spermathecae two, highly chitinised, pyriform, sub-equal; length about  $59\mu$ , greatest breadth about  $43\mu$ , the commencement of the duct chitinised for only a short distance, about  $4\mu$ .

NIGERIA, Southern Provinces: Lagos, November, 1921 (Dr. H. Andrew Foy); collected in the evening upon a lamp-shade. This insect, which is somewhat similarly coloured, may be distinguished from *E. versicolor* by, among other characters, the length of the last antennal segment, the abdominal markings, and the size of the spermathecae: it differs also from *E. africana*, notably in colour. We have pleasure in dedicating this species to the collector, Dr. H. Andrew Foy.

*Ankistrodactylus\** (*Schizodactylus*) *par*, sp. nov.

## MEASUREMENTS.

Length of body (one female)	...	...	...	...	...	4.5 mm.
Length of wing	...	...	...	...	...	2.8 mm.
Greatest breadth of wing	...	...	...	...	...	1.0 mm.

*Head* dark brown, wider than the thorax, flattened from before backwards. Eyes bare, narrowly separated above. Clypeus and proboscis darkish brown, the stylets of the proboscis highly chitinated. Palpi dark brown: first segment rudimentary, second, third and fifth sub-equal, fourth rather smaller; third segment not inflated and without a sensory pit, but bearing a patch of sensory hairs anteriorly, fifth cylindrical, not dilated at its end. *Antennae*: first segment bearing a few hairs; torus dark yellowish-brown, somewhat pyriform, bearing a few hairs; third segment rather longer than the following segments, with a short stalk; segments four to ten pale brown, distal portions of each slightly darker, bearing scanty hairs, sub-cylindrical, the middle slightly wider than the ends, length ranging from about twice to rather over three times the breadth; segments eleven to fifteen elongated, darkish brown excepting the basal sixths, the proximal four seven to eight times as long as broad, the fifteenth slightly longer, nearly ten times as long as broad, with a conical end without a stylet. The combined lengths of segments eleven to fifteen greater than the combined lengths of segments four to ten, namely, about 200 units to 130, or 1.5 to 1. *Thorax* greyish-pruinose, with two longitudinal darker, brownish, stripes on each side, one admedian and the other sub-lateral, the two admedian stripes converging anteriorly. In carbolic acid the thorax appears uniformly very dark brown. Hairs small and scanty. Pleurae dark brown. Scutellum dark brown, bearing numerous bristles and small hairs. Post-scutellum dark brown with a greyish pruinosity. *Wings* (fig. 19) brownish, especially anteriorly; venation and dark markings as shown in the figure. Fringe short. Wing surface covered by microtrichia but without longer, decumbent hairs. Halteres with yellowish- or orange-brown

\* Mr. Edwards has kindly informed us that a new name is required for this genus as *Schizodactylus* is preoccupied. See Scudder's *Nomenclator*.

knobs. *Legs*: femora brown, proximal halves orange-brown, distal halves very dark brown and bearing numerous (a dozen or more) short, stout, black, ventral spines; fore femora slightly broader than the others. Tibiae on fore and middle legs yellowish-brown with infuscated apices and bases; hind tibiae entirely dark brown, and with a regular dorsal row of longish hairs. First four tarsal segments on all the legs pale brown, fifth very dark brown. First tarsal segment longer than the second on all the legs; fourth not cordiform. On the first tarsal segment of the middle legs and the second of the hind legs is a single longitudinal row of small spines; on the first tarsal segment of the hind legs is a double row of similar spines. The fifth tarsal segments on all the legs bear several (five

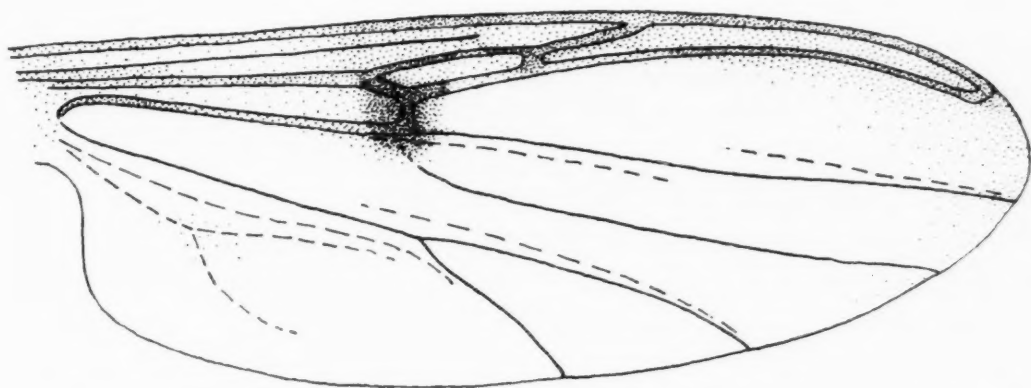


FIG. 19. *Ankistrodactylus* (*Schizodactylus*) *par*, sp.n., wing of female to show adornment and venation (fringe not shown).  $\times$  c. 40.

or six) pairs of short, stout, black spines. Claws on all the legs equal, long, about as long as the fifth tarsal segment, with a rather large basal barb. Empodium rudimentary. *Abdomen* greyish-pruinose dorsally, each segment having at the base a darker, brownish, band which is narrow but expanded a little in the middle line, and bearing two small admedian brown spots on the posterior third; the second segment bears also two larger brown spots near its anterior lateral angles; darkish brown laterally; venter paler brown. Spermathecae two, very highly chitinised, oval, unequal, measuring in one specimen about  $148\mu$  by  $106\mu$ , and  $122\mu$  by  $95\mu$ ; the duct chitinised for only a short distance, about  $7\mu$ , at its commencement.

GOLD COAST: Christiansborg, near Accra, 29th October, 1921; two females, reared from plants of *Pistia stratiotes*.

*Palpomyia pistiae*, sp. nov.

MEASUREMENTS.	Male.	Female.
Length of body (one male and one female) ...	3.6 mm.	5.6 mm.
Length of wing ... ..	2.3 mm.	4.3 mm.
Greatest breadth of wing ... ..	0.65 mm.	1.1 mm.

*Head* very dark brown. Eyes bare, in both sexes separated by a wedge-shaped space which is broadest at the vertex. Clypeus dark brown, hairy. Proboscis dark brown, short, labium fleshy; in the female, mandibles highly chitinated and strongly serrated. Palpi dark brown; first segment rudimentary, second and fourth sub-equal, third longer than the fourth, cylindrical, without a definite pit but with a small anterior depression from which arise a few sensory hairs, fifth segment the longest nearly twice the length of the fourth. *Antennae*: in the female, first segment darkish brown, bearing a few hairs; torus dark yellowish-brown, sub-spherical, bearing a few hairs; segments three to ten dark brown apically and pale brown basally, almost cylindrical, the third slightly longer than the succeeding segments, the fourth to the tenth sub-equal, about three times as long as broad; segments eleven to fifteen entirely dark brown, elongated, length ranging from about eleven to fifteen times the breadth, the last segment not ending in a stylet; the combined lengths of segments eleven to fifteen greater than the combined lengths of segments four to ten, namely, about 451 units to 210, or 2.1 to 1. In the male, torus larger and darker than in the female, basal segments of the flagellum rather pale brown, bearing a well-developed plume of pale brown hairs; segments four to eleven sub-equal and decreasing very slightly in size and gradually becoming darker from base towards the apex; twelfth segment all dark brown, about the same size as the eleventh; segments thirteen to fifteen completely dark brown, elongated, lengths respectively about seven, sixteen, and twenty-five times the breadths, the last segment without a stylet. *Thorax* uniformly very dark brown, bearing very small hairs, and a sharply pointed tubercle projecting forwards from the middle of the anterior margin. Pleurae very dark brown. Scutellum very dark brown, bearing in both sexes numerous short bristles and small hairs. Post-scutellum very dark brown. *Wings* (fig. 20) brownish, with darker markings as shown in the figure. Surface granular, but without either microtrichia or longer, decumbent hairs.



Fringe very short. Venation in the female as shown in the figure; in the male, second cell not so long, the third vein joining the costa a little further from the tip of the wing. Halteres with dark brown knobs. *Legs*: femora and tibiae almost uniformly very dark brown, but in the male the bases of the fore femora and the apices of the fore tibiae rather paler; the femora not swollen, and bearing ventrally on the apical halves of all the legs a few short, stout, black spines—in the male seven or eight on the fore legs and three on the middle and hind legs, in the female nine or ten on the fore, three or four on the middle, and four or five on the hind legs. First two tarsal segments yellowish-brown, third similarly coloured at its proximal end but infuscated distally, the fourth and the fifth entirely dark brown. First tarsal segment at least twice as long as the second on all

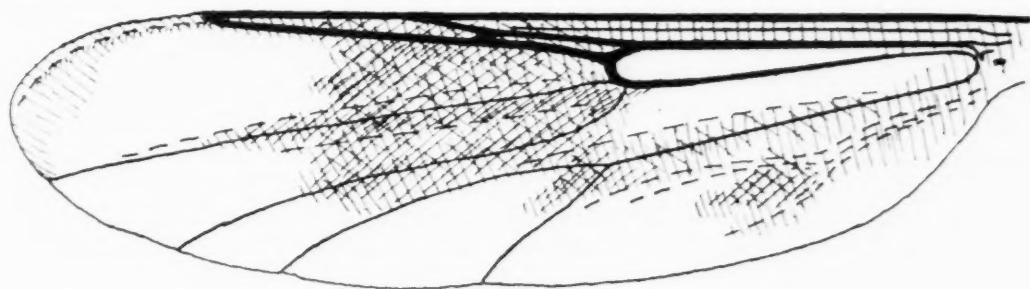


FIG. 20. *Palpomyia pistiae*, sp.n., wing of female to show adornment and venation.  $\times c. 25$ .

legs in both sexes; the fourth cordiform; and in the female the fifth bearing four or five pairs of rather long, black spines. Claws in both sexes about half the length of the fifth tarsal segment, equal in the female, bearing at the base a fairly large barb, and in the male, bifid at the tips. *Abdomen* very dark brown, venter paler than the dorsum; hairs small and scanty. Spermatheca single, very highly chitinised, oval, length about  $125\mu$ , greatest breadth about  $110\mu$ ; the commencement of the duct chitinised for about  $30\mu$ .

**HYPOPYGIUM** (fig. 21). *Ninth segment* very short: tergite short, with two large lateral processes posteriorly which are partially chitinised and bear numerous long and short hairs; sternite very short, moderately excavated in the middle line posteriorly. *Forceps* set almost at a right angle with the long axis of the body, highly chitinised: side-pieces of the usual form; claspers ending in a stout, black, claw-like process. *Harpes*: basal root-like portion very dark



and highly chitinised; distal portion less highly chitinised, directed posteriorly, and expanded at its end as shown in the figures. *Aedoeagus* large, appearing in a ventral view as an oblong structure with short, black, root-like processes and an apical extension bearing on each side a hook-like process. The ventral surface of the

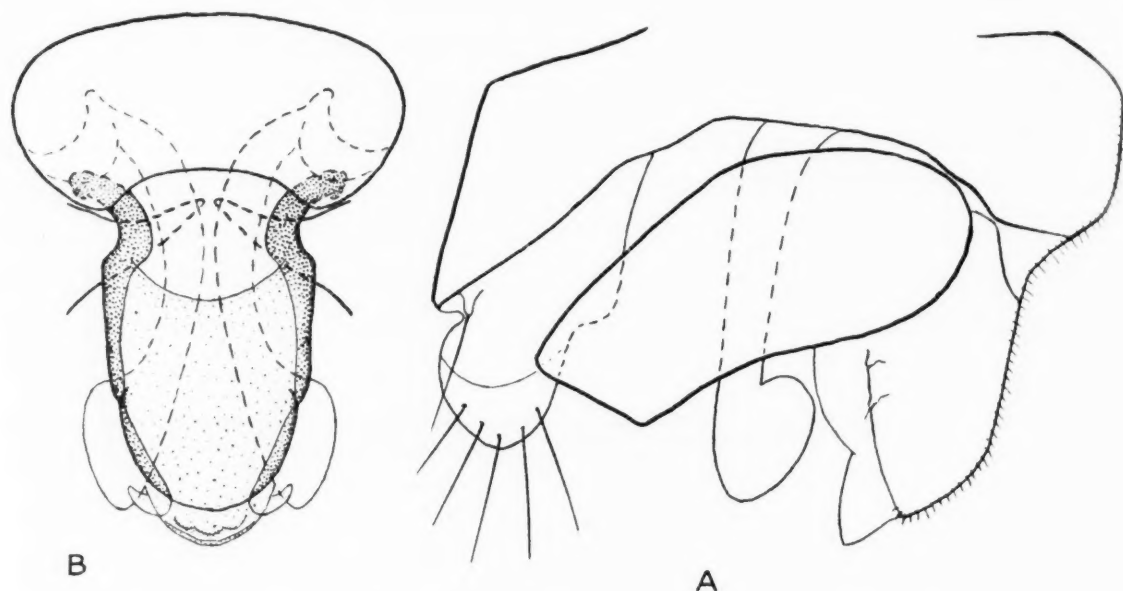


FIG. 21. *Palpomyia pistiae*, sp.n., hypopygium of the male, *A*—lateral view; *B*—ventral view of ninth sternite and median structures only (hairs on ventral surface of aedoeagus not shown).  $\times$  c. 250.

aedoeagus and the membrane joining it to the ninth sternite covered with minute hairs.

GOLD COAST: Nsawam, a town about twenty-five miles north of Accra, 26th February, 1922; one male and one female, reared from plants of the water-weed *Pistia stratiotes*.

*Parabezzia poikiloptera*, sp. nov.

MEASUREMENTS.		Male.	Female
Length of body (one male and one female)	...	2.1 mm.	2.2 mm.
Length of wing	... ..	1.7 mm.	1.7 mm.
Greatest breadth of wing	... ..	0.5 mm.	0.6 mm.

*Head* dark brown; elongated antero-posteriorly, the proboscis directed forwards. Eyes bare; in both sexes separated above by a wedge-shaped space, broadest at the vertex. Clypeus dark brown. Proboscis dark brown; mandibles in the female highly chitinised and strongly serrated, bearing on the inner margin seven stout, triangular teeth. Palpi dark brown, excepting the fifth segment which is

slightly infuscated at the base but is otherwise entirely colourless: first segment very small, about half the length of the fourth, second slightly longer than the fourth, third and fifth longer, sub-equal, nearly one and a half times the length of the second, the third only very feebly inflated in the middle but furnished with a large sensory pit, the fifth somewhat swollen at its end. *Antennae*: in the female, brown, last five segments and the distal thirds of segments three to ten dark brown. First segment darkish brown, rather large, hairless; torus dark yellowish-brown, bearing a few small hairs. Third segment sub-cylindrical, about three times as long as broad; segments four to ten constricted sub-apically, somewhat bottle-shaped, from two and a half to four times as long as broad; segments eleven to fifteen elongated, cylindrical, length increasing progressively from about eight to twelve times the breadth, the last segment being the longest and ending in a blunt process. The combined lengths of segments eleven to fifteen greater than the combined lengths of segments four to ten, namely, about 200 units to 135, or nearly 1.5 to 1. In the male, almost uniformly dark brown (excepting the apices of the thirteenth and fourteenth segments, which are white), and bearing a well developed plume of dark brown hairs. First segment small, hairless; torus large, dark yellowish-brown, bearing a few small hairs; third segment rather large, with a long stalk and bearing two whorls of hairs; segments four to eleven progressively lengthening and narrowing; the twelfth somewhat more produced distally, nearly four times as long as broad; the last three segments elongated, about twelve to fourteen times as long as broad, the fifteenth ending in a blunt process. The combined lengths of segments twelve to fifteen greater than the combined lengths of segments four to eleven, namely, about 195 units to 130, or 1.5 to 1. *Thorax* yellowish-brown mottled with paler, greyish, markings, with dark brown spots at the sockets of the hairs, and with a pale-coloured, median, conical projection anteriorly; sparsely clothed with rather long hairs, which are mostly arranged in five antero-posterior rows, a median, two sub-median, and two sub-lateral. Pleurae rather dark yellowish-brown. Scutellum darkish brown, paler, whitish, posteriorly, especially in the middle line; bearing in both sexes two admedian and two lateral bristles and four small hairs. Post-scutellum dark brown, with greyish anterior

patches. *Wings* (fig. 22) pale, brownish, especially near the anterior margin from the level of the anterior cross-vein to the root of the wing, with numerous small, dark brown markings, mostly restricted to the veins, as shown in the figure. In the male the dark marks are rather smaller than in the female, and the infuscation of the lower ramus of the fifth vein is interrupted in the middle. Venation as shown in the figure. Wing surface covered by microtrichia, but without longer, decumbent hairs. Halteres pale yellowish-brown with dark brown knobs which, however, contain a certain amount of the whitish pigment found also in other parts of the body. There are a few small hairs at the bases of the knobs. *Legs* almost uniformly yellowish-brown, but the distal ends of the tibiae and tarsal segments, and the bases of the first tarsal segments on the

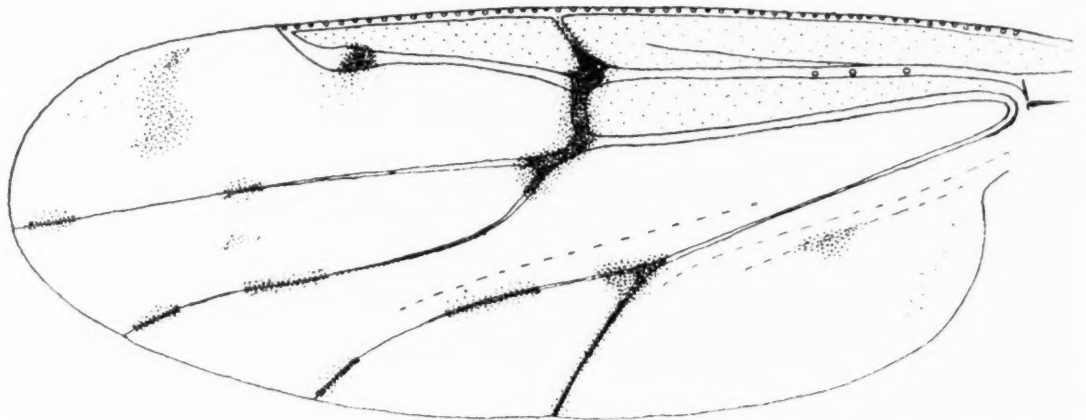


FIG. 22. *Parabezzia poikiloptera*, sp.n., wing of female (fringe not shown) to show adornment and venation.  $\times$  c. 70.

fore and hind legs are slightly darkened, and small infuscated patches can be distinguished about the middles of the fore and hind femora. Femora not armed with spines and not swollen. Tibiae not armed with spines, but on the hind legs bearing a row of about seven rather long, strong bristles on their apical halves. First tarsal segment at least twice as long as the second on all the legs: on the fore and middle legs armed with a strong basal spine, and similar spines one near the middle of the segment and one (or a pair) near the apex; on the hind legs bearing a strong basal spine and three complete longitudinal rows of small spines. Third tarsal segment on all the legs cylindrical, fourth strongly bilobed, fifth not infuscated and not swollen. Claws in the female, single, nearly as long as the fifth tarsal segment, with a large basal tooth; in the male, two, equal,

small, about half the length of the fifth tarsal segment, with bifid tips. Empodium rudimentary. *Abdomen* almost entirely yellowish- or greenish-white dorsally (the colour being due to a pigment soluble in caustic potash but not in carbolic acid) and brown laterally and ventrally; very sparsely clothed with hairs. Spermathecae two, highly chitinised, oval, sub-equal, and rather small; length  $61\mu$  to  $65\mu$ , greatest breadth about  $50\mu$ , the commencement of the duct chitinised for about  $10\mu$ .

**HYPOPYGIUM** (fig. 23). *Ninth segment*: tergite rather short but prolonged posteriorly as two large membranous processes, tapering distally, bearing laterally and posteriorly on its dorsal surface a few

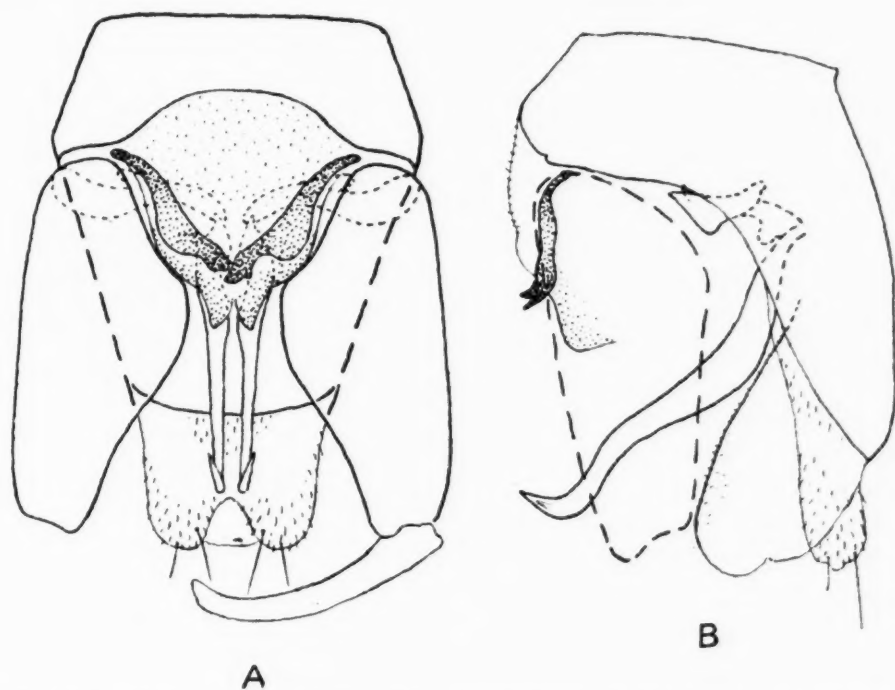


FIG. 23. *Parabezzia poikiloptera*, sp.n., hypopygium of the male, A—ventral view; B—lateral view, the aedoeagus slightly shaded.  $\times$  c. 185.

large hairs, posterior margin rounded, not notched, sternite not very deeply excavated posteriorly. *Forceps* normal in type: side-pieces well developed, tapering distally; claspers rather feebly chitinised. *Harpes* well chitinised: basal portions situated transversely, in part very dark, with a small anterior process on each side; distal portions long, narrow, chitinous bands directed posteriorly and ventrally, tapering distally, and ending in sharp, ventrally-bent, points. *Aedoeagus* with two very strongly chitinised rods, one on each side, directed inwards and backwards, their posterior extremities not fused, but overlapping slightly; these rods have a semicircular

expansion about the middle of their posterior border, and end in a ventrally directed, spine-like process. The membrane joining the aedoeagus to the ninth sternite is spiculated on its anterior three-quarters.

PUPA. Dark brown, highly chitinised; length about 3 mm. to 4 mm. *Respiratory trumpets* (fig. 24 A) peculiarly shaped, with an expanded basal portion, and a long, spine-like, and very dark-coloured distal portion. The main tracheal trunk gives off seven or eight short branches in the expanded portion, and about sixteen to eighteen, arranged in a row along the terminal two-thirds, in the distal portion. *Cephalo-thorax* yellowish-brown, somewhat

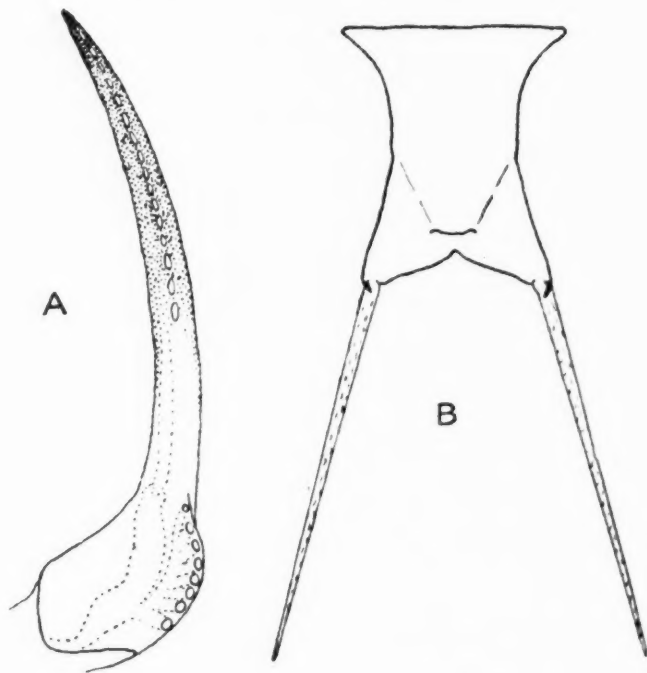


FIG. 24. *Parabazzia poikiloptera*, sp.n. A—respiratory trumpet of pupa.  $\times$  c. 185. B—posterior extremity of abdomen of pupa.  $\times$  c. 90.

infuscated dorsally. Anterior marginal tubercle small, bearing a rather short, stout, bristle; anterior dorsal small, bearing a long hair; anterior dorso-median small, double, each half bearing a small hair; anterior dorso-lateral very large, conical; bearing two long hairs; ventro-lateral ill-defined, bearing a long hair and a socket-like mark; ventro-median obsolete, represented by a small single hair. Dorsal tubercles obsolete, represented by three moderately long hairs. There are on each side of the dorsum also two puckered marks anterior to the situation of the dorsal tubercles, and three socket-like marks more posteriorly. Posterior dorsal tubercle



apparently unarmed. Posterior margin of the dorsum prolonged backwards in the middle line as a conical process. *Abdomen* darker than the cephalo-thorax, infuscated excepting at the bases of the tubercles—which, therefore, by contrast show up as pale elongated areas—reticulated, with small oval or rounded, smooth, pigmented spots arranged as in *Stilobezzia spirogyrae*. First segment short and broad, second longer, third to eighth gradually narrowing, and the ninth small (fig. 24 B), elongated, terminating in two very long lateral spine-like processes which diverge slightly, are spiculated, and bear at their bases two short spines, one dorsal and the other ventral. Tubercles well developed, highly chitinised. Dorsal tubercles: antero-submarginal single, situated anterior to the interspace between the second and third postero-marginal tubercles, small, bearing a moderately long hair; postero-marginal, five, the inner with two prongs and the others single, the inner bearing a small hair, the second and third apparently unarmed, the fourth and outer bearing small hairs. Ventrō-lateral tubercles: antero-submarginal, absent; postero-marginal, three, the ventral small, without a prong, bearing a long, stout, slightly pubescent bristle, the other two larger, with single prongs, the middle bearing a long hair, and the dorsal a short hair. Ventral tubercles: postero-marginal, three, small, the outer two-pronged and bearing a small hair, the other two bearing long hairs.

**LARVA.** The larva is eel-like and almost white; length about 7 mm. or 8 mm., greatest breadth about 0.4 mm. *Head* yellowish-brown, long, rather narrow; length about 0.38 mm., greatest breadth about 0.17 mm. Eyes black, small, composed of two rounded spots of pigment the posterior of which is the larger, situated a little anterior to the middle of the head. Antennae and palpi relatively large; labrum large and bearing numerous small papillae; mandibles powerful, hook-like. Hairs small, arranged as in *Probezia pistiae*. Mental plate apparently with four equal teeth at its anterior margin. Hypopharynx not very strongly chitinised, the posterior sclerite comb-like, bearing on each side about seven pointed teeth. *Body* cylindrical, composed of twelve elongated segments each bearing a few small hairs. On the distal end of the anal segment are fourteen stronger hairs, arranged as follows: dorsally and ventrally two pairs of long, stout, black hairs more than half the length of the anal

segment, and laterally a pair of short hairs on each side, with a very small divided hair between them and slightly more anterior. Anal gills of the usual form, rather short.

GOLD COAST: Accra, April and May, 1922; males and females, reared from plants of the water lettuce, *Pistia stratiotes*, taken from a pool near to the station for the Weshiang Railway Line. The larvae frequent the bases of the *Pistia* plants. The adults are phototropic, and in our breeding jars were commonly found resting on the side towards the light and with their tails directed upwards.

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# THE PATHOLOGICAL EFFECTS PRODUCED BY *STRONGYLOIDES* IN A CHIMPANZEE

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*(Received for publication 13 July, 1922)*

## PLATE XIII

The animal first came under our observation on 11th January, 1922, at which time it was suffering from dysentery. The faeces were examined on the 11th and 12th January, but no ova or larvae were found. On 5th February the animal had a mild attack of diarrhoea, and numerous rhabditiform larvae of *Strongyloides* were found in the faeces; no blood was passed. After 5th February the animal showed no intestinal symptoms of any kind; it remained under our observation for malaria till its death on 23rd February. On February 23rd, at 8.30 a.m., the animal appeared well and made a good meal. At noon the same day it was found lying in its cage in a condition of collapse and breathing with difficulty; it had vomited a large quantity of bile-stained material; death occurred in half an hour.

## POST-MORTEM EXAMINATION

### MACROSCOPIC.

The cause of death appeared to be innumerable small recent haemorrhages uniformly distributed over the whole surface of both lungs (Plate XIII, fig. 1). The only other lesion found

in the lungs was emphysema along the inner margins of the lower lobes of both lungs. The vessels on the surface of the brain were dilated. The pericardium contained about an ounce of fluid. The jejunum, from a point about twelve inches below the pylorus, was thickened throughout its whole circumference for a distance of five inches (Plate XIII, figs. 2-4). In this part the gut wall was about 8 mm. thick, as compared with about 3 mm. in the normal part of the jejunum; the mucosa over the whole affected area was friable. At the commencement of this thickened area there was a conical tumour projecting into the lumen of the gut. The base of this tumour was about 3 cm. in diameter, and its apex projected 1.5 cm. into the lumen of the gut. No other lesions were found in the gut.

#### MICROSCOPIC.

Filariform *Strongyloides* larvae were found in lung smears (Text-fig. 1). Sections of the lung showed emphysema in the

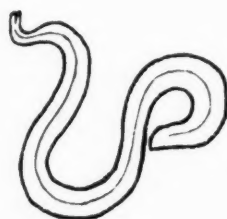


FIG. 1. Filariform larva of *Strongyloides* in the lung.

immediate neighbourhood of the haemorrhages. This emphysema was probably acute following on the haemorrhages and dyspnoea. Filariform *Strongyloides* larvae were found in scrapings of the mucous membrane of the trachea and bronchi. Rhabditiform larvae were present in the trachea, but these were derived from vomited material drawn into the trachea before death. Filariform *Strongyloides* larvae were also discovered in blood from the right ventricle, in the pericardial fluid, in the liver and spleen.

The size of the larvae varied in length from 0.324 mm. to 0.442 mm., and in width from 0.016 mm. to 0.022 mm.

No larvae were found in the brain, and the vascular dilation was probably due to the dyspnoea preceding death.

Examination of sections of the affected part of the jejunum showed thickening due to a large increase in lymphoid tissue in the mucosa, and still more in the sub-mucosa; the muscular layers and serous coat were thickened and showed small-celled infiltration. Innumerable adult worms were present, many projecting into the lumen of the intestine, but the majority were buried deep in the mucosa (Text-fig. 2). In many parts the epithelium of

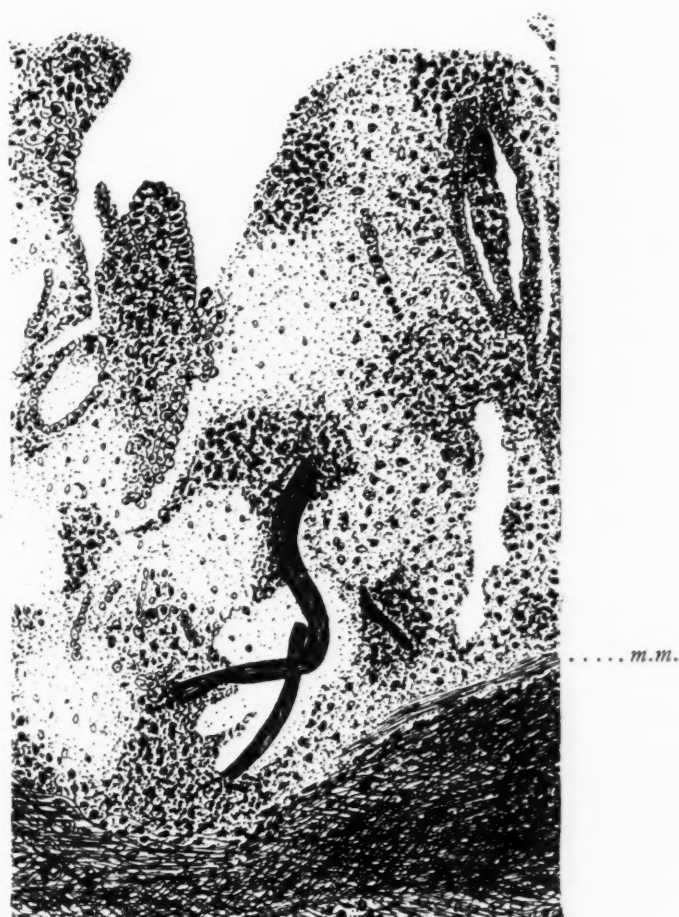


FIG. 2. Adult worm lying above the muscularis mucosae (*m.m.*).  $\times$  c. 300.

Lieberkühn's crypts was destroyed, apparently through the mechanical agency of the worms. Adult worms were also found in the sub-mucosa (Text-fig. 3) down to the level of the circular muscle coat. Empty worm spaces were seen both in the mucosa and in the sub-mucosa. The worms evidently possess the power of moving through the muscularis mucosae, as some were found projecting through it externally into the sub-mucosa and internally into the



mucosa. In spite of the movement of the worms in the tissue, only very few small haemorrhages were found. Ova with developed embryos were present near the surface and throughout the mucous membrane.



FIG. 3. Parts of adult worms and ova above the muscularis mucosae, and of adult worms beneath the muscularis mucosae (*m.m.*).  $\times$  c. 300.

The tumour on section was found to consist of a core of muscle tissue. This core was surrounded by a thick layer of lymphoid tissue extending up to the muscularis mucosae. The mucosa and

lymphoid tissue beneath the muscularis mucosae contained numerous adult *Strongyloides*. Adult worms were found adjacent to the muscular core of the tumour; there is, therefore, evidence here that the presence of *Strongyloides* in the sub-mucosa may cause hypertrophy, and even tumour formation.

Adult *Strongyloides* and rhabditiform larvae were found free throughout the whole alimentary tract from the oesophagus down to the rectum. The presence of free adults is attributed to the friable state of the infected part of the gut, those above the lesion being carried up by the severe vomiting which preceded the death of the animal. The size of the adult worms varied from 1.8 mm. to 2.5 mm. by 0.044 to 0.057 mm.

There are several points of interest in the case of this chimpanzee.

(1) During its attack of dysentery, when it was passing blood and mucus, no larvae were found on two successive days.

(2) In spite of a heavy infection, there was no diarrhoea present from 16th January to 5th February, and from 5th February till its death on 23rd February.

(3) The gross lesions in the jejunum were altogether out of proportion to the signs and symptoms, which were slight. It is probable that in some human infections where symptoms are not marked the lesion in the intestine may yet be gross.

(4) The depth at which the worms were found in the intestinal wall seems to preclude the possibility of affecting the worms by the usual helminthicides administered orally. The cures reported by various authors from time to time depend probably on the fact observed in this case that, even with a very heavy infection and considerable damage to the gut, larvae are not always present in detectable numbers in the faeces. Another possible explanation is that those observers were dealing with a slight infection in which the worms were comparatively superficial.

(5) The animal was kept in a wooden box (4 ft. by 3 ft. by 2½ ft.) with a grating on one side placed in the open air. The box was swept and washed out with water daily. The larvae in sufficient numbers to have caused a fatal invasion must have lodged in the crevices of the moist wood.

(6) The invasion must have gone on for several days since larvae were found both in the heart's blood and trachea.

**SUMMARY**

A chimpanzee which died suddenly was found to have numerous recent haemorrhages in both lungs.

The haemorrhages were found to be due to filariform *Strongyloides* larvae. Larvae were found in the lungs, trachea and bronchi, in the heart's blood, in pericardial fluid, in the liver and spleen.

A heavy infection of *Strongyloides* was found in the jejunum, where a tumour, probably caused through irritation, was present. Adult *Strongyloides* were found at all levels down to the circular muscle layer.



## EXPLANATION OF PLATE XIII

- Fig. 1. Lung : portion of the inferior surface of the lower right lobe, showing many circular haemorrhages.
- Fig. 2. Tumour of the jejunum and part of gut affected by *Strongyloides*. Natural size.
- Fig. 3. Normal thickness of the jejunum of the same animal.
- Fig. 4. Surface view of part of affected gut with portion of the tumour on left. Slightly enlarged.



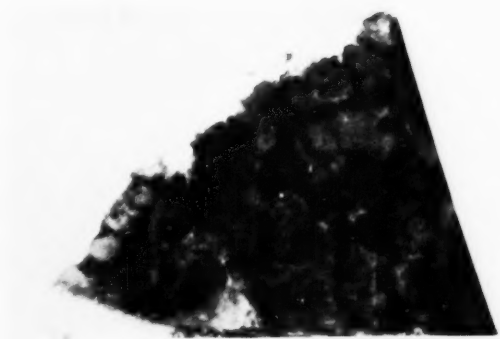


FIG. 1



FIG. 2



FIG. 3



FIG. 4



# PULMONARY LESIONS IN DOGS AND CATS NATURALLY INFECTED WITH NEMATODES

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In an examination of a series of thirty domestic animals, twenty-five dogs and five cats, in Freetown, we observed the constant occurrence of pulmonary lesions. These lesions consisted of:—

(1) Small circular haemorrhages from 1 to 5 mm. in diameter, wedge-shaped on section, occurring on any part of the surface of the lungs. Recent and old discoloured haemorrhages were found in all the animals examined, both in adults and young dogs thirteen and fourteen days old. The number of the haemorrhages varied from one to thirty, except in one domestic cat which was brought into the laboratory ill, and which on examination was found to have numerous haemorrhages in the lungs. The cause of the haemorrhages in this animal was obvious, as numbers of *ancylostome* larvae were found in the bronchi and trachea. In some cases the haemorrhages were situated close together, but were still discrete and never coalesced.

(2) Small scars irregular in their distribution.

(3) Small localised patches of emphysema irregularly distributed over the surface of the lungs, occurring particularly in older animals.

In no case were tubercle bacilli found.

Sections of the haemorrhages showed a variation in the amount of destruction of the interalveolar walls, marked in some and slight in others. The older haemorrhages showed invasion by small

lymphocytes and polymorphonuclear leucocytes, and the presence of fibroblasts. The second type of lesion is probably the result of absorption and fibrosis of old haemorrhages.

All the animals examined were infected with ancylostomes, *A. caninum* or *A. ceylanicum*, or both together, being found. In addition *Toxascaris* and *Belascaris* were found in most of the animals. Although ancylostome larvae were found in the trachea of only five of the thirty animals examined, the haemorrhages in the lungs were, in our opinion, caused by the invasion of nematode larvae. The animals were collected at random from various parts of Freetown, and were apparently average specimens of local animals. The only factors common to all were the presence of nematodes, especially ancylostomes, in the intestine and the lesions in the lungs described, for which no other cause but invasion by nematode larvae could be found. It is noteworthy that in all the animals except the one domestic cat, the number of adult ancylostomes in the intestine exceeded by far the number of haemorrhages in the lungs. It follows, therefore, that the majority of the haemorrhages caused by ancylostome larvae rupturing the capillaries to enter the bronchioles are absorbed without leaving any visible trace, and that the minority are followed by fibrosis, a process which can be followed in sections of some of the haemorrhages.

The small patches of emphysema are due probably to those haemorrhages in which damage to the alveolar walls occurred, and in which fibrosis did not occur along with the absorption of the blood. Owing to difficulties in obtaining post-mortem examinations, we have not been able to ascertain the presence of these lesions in the lungs of human beings. It is obvious that they must occur, since *Ancylostoma* and *Strongyloides* and *Ascaris* must all pass through the lung before reaching their final destination in the intestine. Infection and re-infections with these parasites involving repeated trauma to the lung tissue are common in Freetown and throughout the Tropics. The well-known liability of the native to phthisis has generally been ascribed to lack of acquired immunity, but the constantly recurring trauma to lung tissue by nematode larvae is probably an important accessory factor which will doubtless receive attention from students of tuberculosis in the Tropics.

# ANCYLOSTOMES IN ANIMALS IN FREETOWN

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Yorke and Blacklock (1915) found that dogs in Freetown were heavily infected with ancylostomes. The species found were *A. caninum* (Ercolin, 1859) and *A. ceylanicum* (Looss, 1911), which were present in the intestines in about equal numbers.

During April, 1922, a number of dogs collected from various parts of Freetown were examined in the Laboratory. It was noted that in ten young dogs, two to three months old, *A. caninum* was constantly present, but no specimens of *A. ceylanicum* were found. An examination of ten adult dogs showed, however, that *A. ceylanicum* was present in eight and *A. caninum* was present in

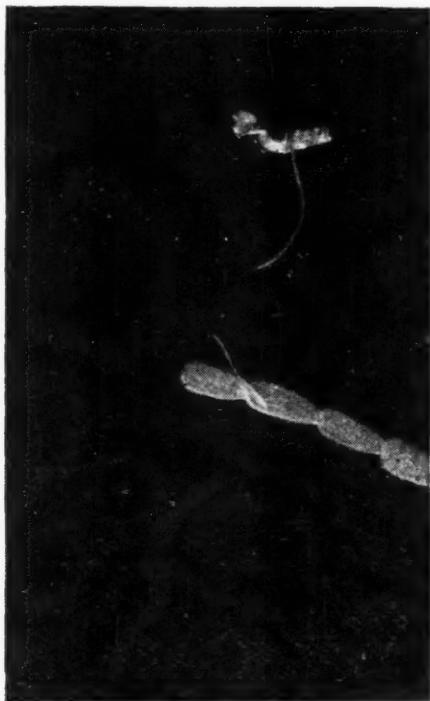


FIG. 1. Two specimens of *Ancylostoma caninum* attached to segments of *Dipylidium caninum*.

every case. It was observed that in dogs *A. ceylanicum* occurs in greatest numbers at a higher level of the intestine than *A. caninum*. In one dog examined a specimen of *A. caninum* was found attached to the mucosa of the large intestine, two inches below the caecum.

Three instances of hyper-parasitism were observed in which



*A. caninum* was found firmly attached to segments of *Dipylidium caninum* (fig. 1).

Six domestic cats, five adults and one kitten, were examined. *A. ceylanicum* was found in the five adults, and of these, three were also infected with *A. caninum*. In one case, specimens of *A. ceylanicum* were found attached to the mucosa of the pylorus. Of three adult specimens of the civet cat, *Viverra civetta*, one was infected with *A. duodenale* and another with *A. ceylanicum*. One genet was examined, and was found to be infected both with *A. caninum* and *A. duodenale*.

Clayton Lane, in India, found that the size of *A. ceylanicum* varied in different hosts. He gives the following measurements:—

Male 5 mm.	...	Female 7 mm.	in the Civet cat
„ 6.8 mm.	...	„ 7 mm.	in the cat
„ 7.2 mm.	...	„ 9.8 mm.	in the dog
„ 8.5 mm.	...	„ 10.5 mm.	in man.

Measurements of *A. ceylanicum*, *A. caninum* and *A. duodenale* in the hosts recorded above gave the following results:—

		Dogs	Cats	Genet	Civet Cat
<i>A. ceylanicum</i> ...	♂	6.0—8.0 mm.	6.0—8.5 mm.	...	5.2—6.2 mm.
	♀	7.0—10.0 mm.	7.0—11.0 mm.	...	6.2—6.8 mm.
<i>A. caninum</i> ...	♂	6.5—9.0 mm.	7.0—8.5 mm.	5.3—9.2 mm.	...
	♀	7.5—14.0 mm.	8.0—13.0 mm.	5.3—13.4 mm.	...
<i>A. duodenale</i> ...	♂	...	...	5.5—5.8 mm.	...
	♀	...	...	5.8—6.8 mm.	8.0—10.0 mm.

All the females included in the measurements were mature.

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# THE OCCURRENCE OF ANCYLOSTOMES RESEMBLING *NECATOR AMERI- CANUS* AMONGST DOMESTIC PIGS IN AMAZONAS

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O'Connor (1920) records that when examining domestic pigs from Funafuti (Ellice Islands), he found ancylostomes apparently identical with *A. duodenale* of man. 'The larger females were a little more than 0.8 cms. in length, being thus smaller than the human parasite.'

Maplestone (1921) examined one hundred and eighty-two pigs in the Townsville district, with negative results.

Dr. Maplestone has recently shown me four ancylostomes obtained from a pig at Townsville, some time after his paper of 1921 was published. Three of these appear to be *A. duodenale*, 2 ♂♂, 1 ♀. The other is a female ANCYLOSTOMINAE measuring 0.9 cm., and exactly resembling the species described below.

Legg and Rheuben (1921) found nematodes 'closely resembling *A. duodenale* (man)' in three of a small number of pigs autopsied at Cromarty, about twenty miles from Townsville.

With a view to establishing whether such parasites also occurred in Brazilian pigs, the author carried out a series of fifteen post-mortems on pigs from the town of Manáos in Amazonas.

All the animals were of the domestic variety, and had been kept in, or around, native dwelling-houses. Their ages varied from young 'sucking pigs' to full-grown adults.

The intestines were opened on large flat dishes; all nematodes obtained from the gut, or by subsequent washings of the gut contents, were cleaned by shaking in normal saline, killed with hot

75 per cent. alcohol, and stored in Lacto-phenol. The ancylostomes were then separated and a microscopical examination made of each.

No *A. duodenale* were found, but one hundred and seventy-five ANCYLOSTOMINAE (118 ♀♀, 57 ♂♂), corresponding with *Necator americanus* in all respects, except that of size, were collected from the small intestine of ten of the fifteen pigs examined. The largest number obtained from any one animal was seventy-five, the smallest, one.

*Shape and size.* All of the one hundred and seventy-five worms examined showed the S-shaped curve characteristic of *Necator americanus*. The length of twenty-eight males varied from 6.5 mm. to 4.5 mm., average 5.1 mm.; and that of sixty-four females from 8.2 mm. to 5.5 mm., average 6.5 mm. The greatest breadth in the males averaged 230 $\mu$ , and in the females 270 $\mu$ .

*Mouth.* The minute anatomy of the mouth was indistinguishable from that of *Necator americanus*, and the average dimensions of the anterior opening of the mouth capsule in fourteen males was 60 $\mu$  in the dorso-ventral diameter, by 50 $\mu$  in the lateral, and 68 $\mu$  by 57 $\mu$  in the case of thirty-one females.

*Bursa.* The bursal formula differed in no respect from that of *Necator americanus*. Length of spicules 0.48 mm. (average of 12).

*Vulva.* This was situated in the anterior half of the body. It is important to note that some of the females were gravid.

### SUMMARY

Of fifteen domestic pigs examined for ancylostomes in Amazonas, 75 per cent. showed an infection with what is in all probability *Necator americanus*. Such a high proportion of infection would suggest that the pig, in this locality at any rate, plays a part of some importance in the spread of ancylostomiasis.

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## PARASITES IN DOGS AND CATS IN AMAZONAS

BY

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The systematic destruction of stray dogs and cats in Manaus provided us with the opportunity of making post-mortem examinations of fifty dogs and nine cats. Nothing is known of the age of these animals. It was noticed that those in a poor and emaciated condition showed more intestinal parasites than the others. The work was chiefly concerned with ancylostome infection, but other observations were also made as recorded in the tables.

### METHOD OF EXAMINATION

The post-mortem examinations were carried out at the local refuse destructor, where the animals were killed by the fumes from burning sulphur. An advantage of this method is that ectoparasites are killed *in situ*. This was always done during the afternoon, and the animals examined immediately afterwards. After searching for ulcers of a possible *Leishmania* character, all fleas, lice, etc., were collected, and films were made from heart blood, lung and spleen. The stomach and intestines were removed and taken to the laboratory, where they were carefully examined along with the washings from the gut contents. A specimen of faeces from the rectum was taken for microscopical examination. Apart from the intestines and heart, nothing of interest was found in the other organs examined.

## RESULTS

The results of examination of all the material collected are recorded in the tables below. All the ancylostomes in each animal were not examined, as some animals contained many hundreds, but a sample was taken from each case. The total number examined was nine hundred and thirteen, and comprised:—*A. caninum*, 260 ♂♂, 461 ♀♀; *A. braziliense*, 69 ♂♂, 123 ♀♀. The smallest number found in any animal was one, and the largest number examined fifty-eight. Each worm was identified by microscopical examination.

TABLE I.

Showing the results of examination of Dogs and Cats for Ectoparasites.

	No. of animals examined			
	Dogs 50 (34 ♂♂, 16 ♀♀)		Cats 9 (5 ♂♂, 4 ♀♀)	
	No. of animals harbouring	No. of parasites found	No. of animals harbouring	No. of parasites found
<i>Ctenocephalis canis</i> ... ..	11	13	4	7
<i>Trichodectes latus</i> ... ..	3	16	0	0
<i>Heterodoxus longitarsus</i> * ... ..	2	2	0	0
<i>Rhipicephalus sanguineus</i> ... ..	2	4	0	0

\* We are indebted to Miss A. M. Evans for the identification of this parasite.

TABLE II.

Showing the results of examination of Dogs and Cats for Helminths.

	No. of animals examined	
	Dogs 50	Cats 9
	No. of animals harbouring	No. of animals harbouring
<i>Ancylostoma caninum</i> , small intestine ... ..	49	5
<i>Ancylostoma caninum</i> , large intestine ... ..	22	0
<i>Ancylostoma braziliense</i> , small intestine ... ..	37	5
<i>Ancylostoma braziliense</i> , large intestine ... ..	8	0
<i>Belascaris marginata</i> ... ..	4	0
<i>Belascaris cati</i> ... ..	0	4
<i>Toxascaris canis</i> ... ..	3	0
<i>Dipylidium caninum</i> ... ..	10	0
<i>Dirofilaria immitis</i> * ... ..	2	0

\* In the right side of the heart. In the case of another dog, a Nematode belonging to the FILARIIDAE, not yet identified, was found in the peritoneal cavity.



TABLE III.

Showing the results of examination of Faeces of Dogs and Cats.

	No. of animals examined	
	Dogs 50	Cats 9
	No. of animals infected	No. of animals infected
<i>Ancylostome ova</i> ... ..	49	4
<i>Ascaris ova</i> ... ..	2	1
<i>Cestode ova</i> ... ..	1	0
<i>Trichuris ova</i> ... ..	1	0
<i>Lambliia cysts</i> ... ..	3	2
<i>Isospora bigemina</i> ... ..	1	5*

\* Four of these cats had been kept together.

## EXAMINATION OF SMEARS

A nasal ulcer was found in one dog only, the examination of which for *Leishmania* proved negative.

Giemsa-stained smears from lung, spleen and heart blood were examined from each of the fifty dogs and nine cats. Except that some blood films showed eosinophilia, and in the case of one dog a lymphocytosis, the results were negative.

A dog examined some months previous to the fifty here recorded showed microfilaria in the blood, but no adult FILARIIDAE were found.

## SUMMARY

It will thus be seen that all the dogs examined were infected with ancylostomes, *A. caninum* being found in 100 per cent. and *A. braziliense* in 74 per cent. Yorke and Blacklock (1915) from the examination of seven dogs, stated that dogs in Freetown were heavily infected with ANCYLOSTOMINAE, *A. caninum* and

*A. ceylanicum* being present in about equal numbers. Hall (1917) records 'hookworms' in 71 per cent. of seventy-six dogs in Washington, and *A. caninum* in 34 per cent. of sixty-seven dogs in Detroit.

Of the nine cats examined by us, 66 per cent. were infected with *A. caninum*, *A. braziliense*, or both.

As recorded elsewhere, *A. braziliense*, although common in dogs in Manáos, does not occur often in human beings, one of us (R.M.G., 1922) finding only four infections in sixty-seven human post-mortems.

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# A NOTE ON THE PREVALENCE OF CERATOPOGONINE MIDGES ON THE WINDOWS OF THE ACCRA LABORATORY DURING A COMPLETED YEAR

BY

A. INGRAM

AND

J. W. S. MACFIE

(Received for publication 3 August, 1922)

With a view to determining the prevailing species of 'sand-flies' in Accra, the collection of small flies from the windows of the laboratory in the evening was begun in October, 1919, and upon finding that they were present in large numbers, was carried on in a systematic manner for twelve consecutive months, namely, from the 1st of December, 1919, to the 30th of November, 1920.

The method of capture of these small insects was that previously described in the first part of our 'Observations on the Ceratopogonine Midges of the Gold Coast' (*Ann. Trop. Med. and Parasitol.*, XIV, p. 189). The windows of the laboratory, which were open all day, were closed each evening at 5 p.m.: shortly afterwards insects began to appear on the insides of the window-panes, and were secured. The period of collection each evening was usually limited to about an hour, 5.30 to 6.30 p.m., owing to the rapid onset of darkness, but occasionally a few specimens were taken later, in bungalows upon walls in the vicinity of a lamp.

A large and varied collection of small insects was obtained in this way. The number of 'sand-flies' taken, that is Ceratopogoninae and *Phlebotomus* spp., was between three and four thousand, and included several new species, the majority of which have already been described. Specimens of *Culicoides*, it may be noted, are easily taken on glass, as they rarely attempt to fly, differing

in this respect from *Phlebotomus* and from the larger species of *Forcipomyia*.

If it be justifiable to draw conclusions from an exceptionally dry year (see Table I), there would appear to be some seasonal variation

TABLE I.  
Rainfall and Mean Temperature at Accra during the year.

										Rainfall in inches	Mean temperature
1919											
December	...	...	...	...	...	...	...	...	...	...	78°98° F.
1920											
January	...	...	...	...	...	...	...	...	...	0°04	80°43
February	...	...	...	...	...	...	...	...	...	0°18	81°34
March	...	...	...	...	...	...	...	...	...	0°74	84°70
April	...	...	...	...	...	...	...	...	...	3°19	84°66
May	...	...	...	...	...	...	...	...	...	2°12	83°33
June	...	...	...	...	...	...	...	...	...	5°07	81°06
July	...	...	...	...	...	...	...	...	...	...	79°40
August	...	...	...	...	...	...	...	...	...	0°17	78°77
September	...	...	...	...	...	...	...	...	...	0°36	75°58
October	...	...	...	...	...	...	...	...	...	1°36	76°01
November	...	...	...	...	...	...	...	...	...	1°79	79°16

in the prevalence of the species of *Ceratopogonine* midges encountered upon the windows of the Accra laboratory. *Culicoides* were more plentiful than *Forcipomyia* from the beginning of December to the end of May, while from the beginning of June to the end of November they were very scanty. *Forcipomyia*, on the contrary, were rare when *Culicoides* were abundant, and were present in much larger numbers than *Culicoides* in the collections made from the beginning of June to the end of November.

A table (Table II) is given which shows approximately the seasonal prevalence of the more common midges captured. Only those species are included which occurred with tolerable frequency at some part of the year at any rate. In addition, however, single, or but one or two specimens, were taken of the following species:—

*Thysanognathus*\* (*Prionognathus*) *maculipennis*, C., I. and M., *T. maculithorax*, C., I. and M., *T. pseudomaculipennis*, C., I. and M., *Dasyhelea flavipicta*, I. and M., *Atrichopogon acosmetum*, I. and M., *A. africanum*, I. and M., *A. chrysospherotum*, I. and M., *A. elektrophaeum*, I. and M., *A. kelainosoma*, I. and M., *A. perfusum*, I. and M., *A. xanthoaspidium*, C., I. and M., and *Forcipomyia* (*hirsuta*). With regard to the names of species of *Forcipomyia* in the table, it is to be noted that in some cases they are only provisional since they refer to new species, descriptions of which have been written but have not yet been published—such names are indicated by being enclosed in brackets. Species of *Phlebotomus* are not included, since they were all forwarded to Prof. R. Newstead.

From time to time flies of large size were taken whilst searching for 'sand-flies,' but as a rule they were avoided since in their death throes they were apt to damage their more delicate neighbours in the killing tube; for the same reason mosquitoes also were looked upon with disfavour. Although, therefore, no systematic collections of such insects were made, the following facts may be noted. Several specimens of *Auchmeromyia luteola*, F., and *Cordylobia anthropophaga*, Grünb., were taken, and one or two specimens of *Stomoxys nigra*, Macq., but no specimen of *Glossina*—a fact which is not surprising considering the rarity of tsetse-flies in Accra and the lateness of the hour at which the collecting was done. The commonest mosquitoes captured were *Stegomyia fasciata*, F., *Culex fatigans*, Wied., *C. decens*, Theo., and *Ochlerotatus irritans*, and it may be recorded that two specimens of *C. rima*, Theo., and a single specimen each of *Stegomyia luteocephala*, Newst., and of *Culex* (*Micraedes*) *inconspicuus*, Theo., were also taken. These insects presumably were attempting to escape from the laboratory when they were captured.

\* For change of name see *Ante* p. 244.



TABLE II.

The seasonal prevalence of Ceratopogonine Midges on the windows of the Accra Laboratory.

	December, 1919	January, 1920	February	March	April	May	June	July	August	September	October
<i>Culicoides accraensis</i> , C. I. & M.	... xx	x	xx	xx	x	x	...	...	...	...	...
<i>C. austeni</i> , C. I. & M....	... ..	xx	xx	xx	x	x	x	x	xx	x	x
<i>C. citroneus</i> , C. I. & M.	... x	x	x	x	x	...	...	...	...	...	...
<i>C. clarkei</i> , C. I. & M.	... x	xx	x	x	x	x	...	...	...	x	...
<i>C. distinctipennis</i> , Aust.	... x	xxx	x	xxx	...	...	xx	xx	xx	x	x
<i>C. grabami</i> , Aust.	... ..	x	x	x	x	x	...	...	...	...	...
<i>C. neavei</i> , Aust.	... ..	x	...	x	x	...	x	...	...	...	...
<i>C. pallidipennis</i> , C. I. & M.	... x	x	x	x	x	x	x	...	...	...	...
<i>C. schultzei</i> , (End.)	... ..	xx	xxxx	xx	xxxx	xxxx	xxx	xxx	xxx	xxx	xx
<i>C. similis</i> , C. I. & M.	... ..	xx	xx	xxx	xx	...	...	...	...	...	...
<i>Thysanognathus</i> * <i>marmoratus</i> , C. I. & M.	... ..	x	x	x	x	x	...	x	...	...	...
<i>Centrochynchus</i> [ <i>Lasiobelea</i> ] ( <i>inconspicuus</i> )	... ..	...	...	x	...	...	...	...	x	...	x
<i>Forcipomyia castanea</i> , Walk.	... x	x	x	x	x	xxx	xxx	xxx	xxx	xxx	xx
<i>F. incomptifeminibus</i> , Aust.	... ..	...	...	x	...	...	x	...	...	...	x
<i>F. ingrami</i> , Cart.	... ..	xx	xxxxx	xxx	xxxxx	xxxx	xxxx	xxx	xxx	xxx	xxx
<i>F. inornatipennis</i> , †Aust.	... ..	...	...	...	x	x	x	x	x	x	x
<i>F. (biannulata)</i>	... ..	x	...	...	...	x	x	x	x	x	x
<i>F. (squampennis)</i>	... ..	x	x	x	xx	x	xx	xxx	xx	xx	x

x = under ten specimens taken; xx = over ten but under twenty, etc.

\* For change of name see *Ante* p. 244.

† This name possibly includes two species.

## NOTES ON AUSTRALIAN CESTODES

BY

P. A. MAPLESTONE

*(Received for publication 5 August, 1922)***VI. SCHIZOTAENIA CACATUAE, sp. nov.**

This cestode was found in two individuals of the species *Cacatua galerita*, Lath., the common sulphur-crested white cockatoo. One of these was shot on the mainland near Townsville, North Queensland, and the other on Magnetic Island; but as this island is quite close to the mainland, and birds frequently fly from one to the other, the difference in locality is of no importance.

**EXTERNAL ANATOMY.**

The length of fixed specimens is up to 200 mm., and the maximum breadth 5 mm. On the whole, the segments are broader than long, but in the anterior immature portion some proglottides longer than broad are found. These longer segments are not regularly placed, and alternate irregularly with ones that are broader than long. When it occurs, the increase in length is not at the expense of breadth, so that the gradual uniform increase in width is not interrupted. Macroscopically, along the median axis of the chain there is frequently a line of depressions, one in each segment; these taken together form a longitudinal groove. This groove is most marked about the central portion of the worm. The posterior angles of the segments scarcely project beyond the edges of the ones immediately succeeding them, and as development is very slow the result is that proglottides are almost rectangular in shape.

*Head.* The scolex (fig. 1) is almost flat anteriorly, with no rostellum, and has a maximum breadth of 0.240 mm. through the centre of the suckers. The four suckers are placed close to the anterior end, they are flat and circular and do not stand out from the surface; they measure about 100 $\mu$  in diameter, and look outwards and very slightly forwards.

*Segments.* Passing posteriorly, the worm becomes gradually narrower and is unsegmented for a distance of 0.8 mm., at which point it is only 0.130 mm. broad.

At first the young proglottides are all broader than long, but when about 0.5 mm. broad many of them may be longer than broad, as detailed above. At this stage the developing reproductive organs can be clearly seen, and it is thus early apparent that the genital pores are unilateral, opening on the right side (fig. 2).

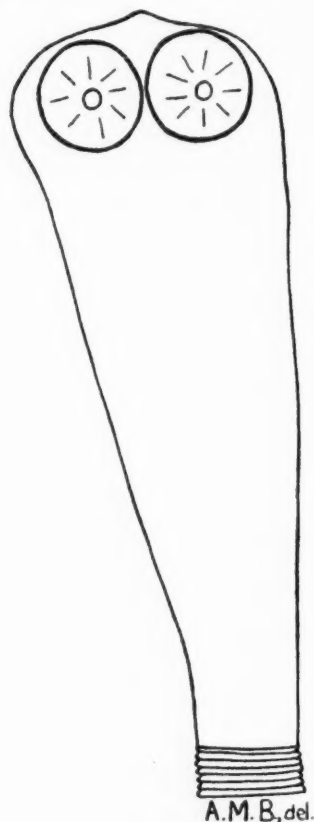


FIG. 1

FIG. 1. *S. cacatuae*. Scolex.  $\times 95$ .

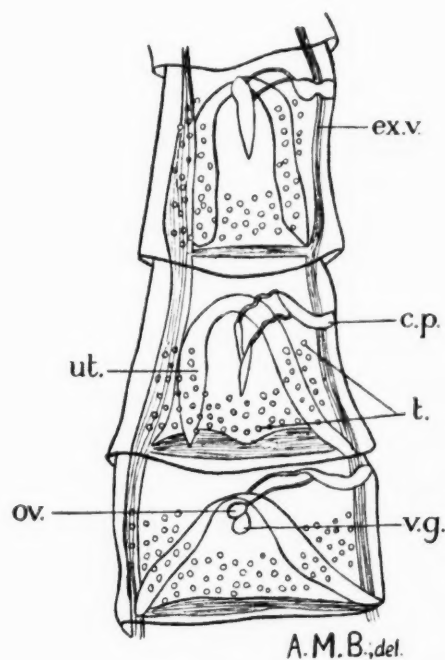


FIG. 2

FIG. 2. *S. cacatuae*. Young proglottides. *c.p.*—cirrus pouch; *ex.v.*—excretory vessels; *ov*—ovary; *t*—testes; *ut*—uterus; *v.g.*—vitelline gland.  $\times 27$ .

Sexually mature proglottides in fixed and mounted specimens measure about 4 mm. broad and 1 mm. long.

#### INTERNAL ANATOMY.

*Muscular system.* The longitudinal muscle is disposed in a single stout layer. Internal to this a thin layer of transverse muscle fibres occurs. Dorso-ventral fibres are fairly well developed.

*Nervous system.* This was not investigated.

*Excretory system.* The lateral excretory vessels lie well towards the lateral borders, and pass ventral to the cirrus pouch and vagina. The dorsal vessel lies on the outer side of the ventral, the latter being joined by a transverse commissural vessel, which runs across the posterior of each segment. The vessels present many variations in

different segments. These variations are most easily seen in young proglottides, and the chief departures from the normal are as follows. The ventral vessel may suddenly dilate about the centre of its course through a segment, and the ends remain of normal calibre, thus forming a fusiform sac. Another common variation is a dilatation into a circular cavity at the junction of the ventral and transverse commisural vessels on each side, which dilatation may extend for some distance along the transverse vessel. Also there is occasionally a branch extending inwards and forwards, from the postero-lateral expansion just mentioned, but in no instance could a connexion between the dorsal and transverse vessels be observed.

*Genitalia.* Development of the sexual organs is slow, and over one hundred proglottides are found, with the sexual organs sufficiently developed to be clearly distinguished before the uterus contains any eggs, and long after this organ has begun to fill the sexual organs remain quite distinct, their atrophy being proportionally as slow as their development.

*Testes.* The testes are large and very numerous, there being over one hundred in each segment. They occupy practically the whole antero-posterior field of the medulla on each side of the ovary. These two groups of testes are united by a bridge of the same glands passing posterior to the ovary. They lie only very slightly dorsal to this organ. The group on the aporal side is somewhat more numerous than the one on the pore side, and it extends dorsal to, and in some cases overlaps the excretory vessels. The testes in the lateral groups are relatively large oval bodies measuring about  $200\mu$  by  $0.30\mu$ , but those more centrally placed, which form the post-ovarian bridge, are smaller (about  $100\mu$ ) and more circular in outline (fig. 3).

*Vas deferens.* Separate vasa efferentia could not be distinguished, but the vas deferens is seen passing dorsal to the ovary; it runs at first forwards and then it curves laterally to the right running transversely across the right anterior portion of the segment. There is no vesicula seminalis. The cirrus pouch lies dorsal to the excretory vessels and nerve, and on a plane slightly dorsal of the testes. When fully developed it is an elongate sac lying transversely in the right anterior quadrant of the segment measuring about  $300\mu$  long by  $130\mu$  broad, and having a bluntly rounded mesial extremity.

As a rule, in the mesial portion of the sac a few coils of the vas deferens can be made out. The cirrus, when extruded, measures about  $650\mu$  long, and is slightly thicker at the base than the apex, and its outer surface is thickly covered with spines. The atrium is about  $160\mu$  deep and  $60\mu$  in diameter, and it opens on the external surface near the right anterior corner of the segment, its external opening having thick everted lips.

*Ovary.* The ovary is practically in the mid line and lies towards the anterior surface of the segment, it is composed of a number of discrete lobes radiating fan-wise. It is slightly asymmetrical, as

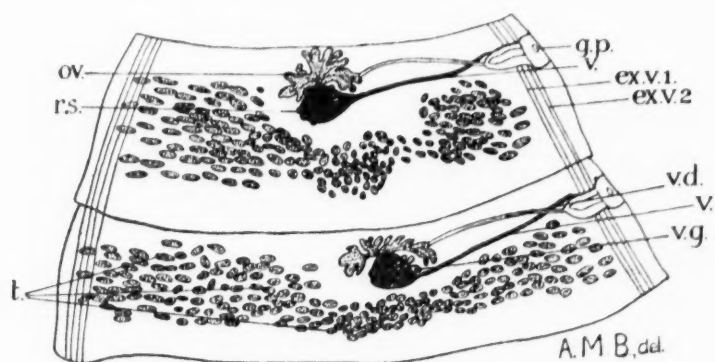


FIG. 3. *S. cacatuæ*. Ripe proglottides. *ex.v.1.*—ventral excretory vessel; *ex.v.2.*—dorsal excretory vessel; *g.p.*—genital pore; *ov.*—ovary; *r.s.*—receptaculum seminis; *t.*—testes; *v.*—vagina; *v.d.*—vas deferens; *v.g.*—vitelline gland.  $\times 17$ .

there are a few more lobes on the aporal than on the pore side. The ducts from the various lobes run centrally, and enter a common duct, behind the centre of the gland. This duct runs posteriorly, and just before it joins the duct from the vitelline glands is surrounded by the shell gland (fig. 3).

*Vitelline glands.* The vitellarium is of similar structure to the ovary, being composed of a number of discrete lobes, which are united by ducts meeting in a point just in front of its centre. It is more or less divided into right and left halves, the larger of which is on the right side. It lies immediately behind the centre of the ovary.

*Receptaculum and vagina.* The vagina opens antero-ventral to the cirrus and passes inwards along the anterior border of the cirrus sac, slightly ventral to it. It crosses the vas deferens ventrally and runs parallel to it to enter the receptaculum seminis which lies dorsal to the vitelline and shell glands. The duct from the receptaculum



to the oviduct cannot be made out, as the former organ completely overlies this part of the field.

*Uterus.* The uterus is visible at a comparatively early stage, and is at first seen as a relatively wide, horse-shoe-shaped tube curving round the ovary anteriorly, and terminating on each side near the posterolateral angles (fig. 2). When eggs first begin to appear in it, they are situated in the two extremities only, and soon subsidiary pouches

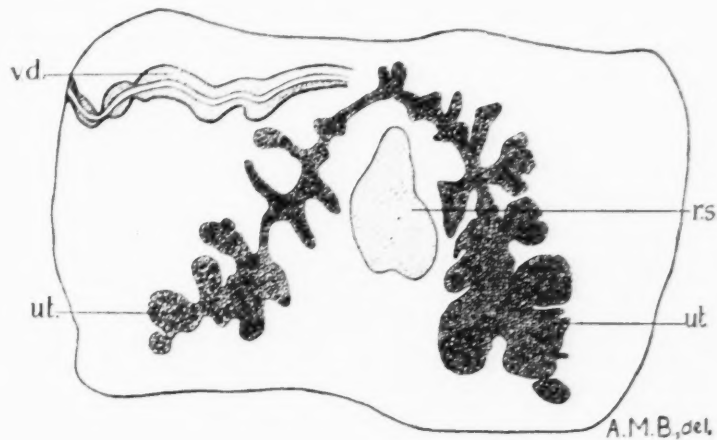


FIG. 4. *S. cacaetuae*. Uterus partly developed. *rs.*—receptaculum seminis; *ut*—uterus; *vd.*—vas deferens.  $\times 17$ .

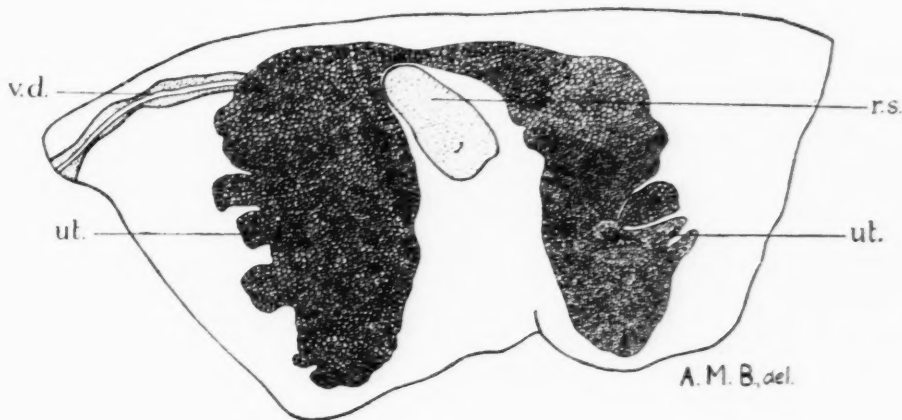


FIG. 5. *S. cacaetuae*. Uterus more fully developed. *rs.*—receptaculum seminis; *ut*—uterus; *vd.*—vas deferens.  $\times 17$ .

are thrown out from it both internally and externally, so that its original tubular structure is lost, except in the mesial part in front of the ovary, where the development of subsidiary branches is never marked (figs. 4 and 5). The most advanced stage of development of the uterus which was observed is shown in fig. 5, here the uterus is seen as two large branching sacs occupying practically the whole

of the lateral fields united anteriorly by a narrow tubular portion. The receptaculum seminis persists throughout, and also remains of the vitellarium can be made out for a long time after all other sexual organs have disappeared. These lie in a central position, and the uterus bends round them. The degenerate reticular nature of the uterus described by Douthitt (1915) was not apparent in our specimens.

*Eggs.* No mature eggs were seen.

#### DIAGNOSIS.

This species agrees with the genus *Schizotaenia* as described by Ransom (1909), except that the genital pores are unilateral.

As far as the writer is aware, this is the first member of the genus to be described from Australia, and still more important, it is the first *Schizotaenia* to have ever been found in a bird, all those hitherto described being from mammals. But other genera of the sub-family *Anoplocephalinae* have been recorded from avian hosts.

The name *Schizotaenia cacatuae*, after its host *Cacatua galerita*, is suggested.

Type specimens of this cestode are in the Museum of the Liverpool School of Tropical Medicine.

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THE *ASCARIS* OF CATTLE

BY

J. W. S. MACFIE

*(Received for publication 8th August, 1922)*

In January, 1921, I received from Major W. P. Beal, Principal Veterinary Officer, Gold Coast, some specimens of *Ascaris*, obtained at Kumasi from a calf, which differed in certain important points from *A. vitulorum* as described by Ransom (1911). In a recent paper entitled 'On *Ascaris vitulorum*, Goeze,' Boulenger (1922) has given an excellent description of the *Ascaris* of cattle based on materials from the Punjab and from Northern Rhodesia, and has pointed out that it fails to agree with the specific diagnosis generally accepted in two most important characters, the worms examined by him possessing cephalic papillae on the lips and post-anal papillae on the tail of the male. As these were also the chief differences noted in the worms collected at Kumasi, I have re-examined my material and have compared it with the description given by Boulenger. The worms were found to be similar in most respects, but certain differences were noted and are briefly described here.

Six males and six females were examined. The length of the males was 10 to 11 cm., and of the females 15.5 to 23 cm. Cuticle with transverse striations, about  $60\mu$  to  $90\mu$  apart in the middle of the body. Head as described by Boulenger, but smaller, as the worms were smaller. Dentigerous ridges well developed, the teeth about  $7\mu$  apart. Oesophagus as described by Boulenger.

*Male.* Posterior extremity as described by Boulenger. But pre-anal papillae about thirty to forty in a row on each side, the more anterior ones being smaller and more widely spaced than those nearer to the cloaca. Large double post-anal papillae, as described by Boulenger, immediately behind the cloaca. Mucronate appendix with two pairs of papillae on its ventral aspect and two pairs on its dorsal aspect in a similar position, but slightly more lateral, Spicules sub-equal, as shown in fig. 2, and much as described by Boulenger; length about 0.9 mm.

*Female.* Posterior extremity as described by Boulenger. Tail short, conical; the distance from the anus to the tip of the tail about 0.7 mm. Vulva situated about 25 mm. from the anterior extremity in a worm about 16.5 cm. long. Vagina composed of two portions which merge gradually; the first portion about 12 mm. long, and narrow, diameter about 0.3 mm., and the second about 10 mm. long, broad, and dividing at its distal end so as to form the two uteri.



FIG. 1. *Ascaris* of Cattle. Head, anterior view,  $\times 75$ . *d.l.*—dorsal lip; *p.*—papilla *d.*—dentigerous ridge.

The uteri run posteriorly, parallel, gradually narrowing and eventually merging with the oviducts. There was no ampulla on the oviducts near their junctions with the uteri. Eggs from the vagina about 0.08 mm. long by 0.06 mm. wide.

The worms, therefore, differ most notably from those described by Boulenger in the number of the papillae on the posterior extremity of the male, and as it is most unlikely that so careful and experienced a worker as Boulenger should have overlooked any of these, it would appear that there are at least two species of *Ascaris* found in cattle in Africa. Further study will be necessary to settle the question whether either of these is identical with the European form.

It may be noted finally that this parasite is the cause of serious disease in the Gold Coast as in Northern Rhodesia. The calf from

which the specimens were obtained died from obstruction of the bowel, and after death, Major Beal found the worms 'in thousands, all intertwined, in the small and large bowels.' The calf was only three weeks old at the time of its death, a fact which is of some interest in view of the possibility of pre-natal infection.

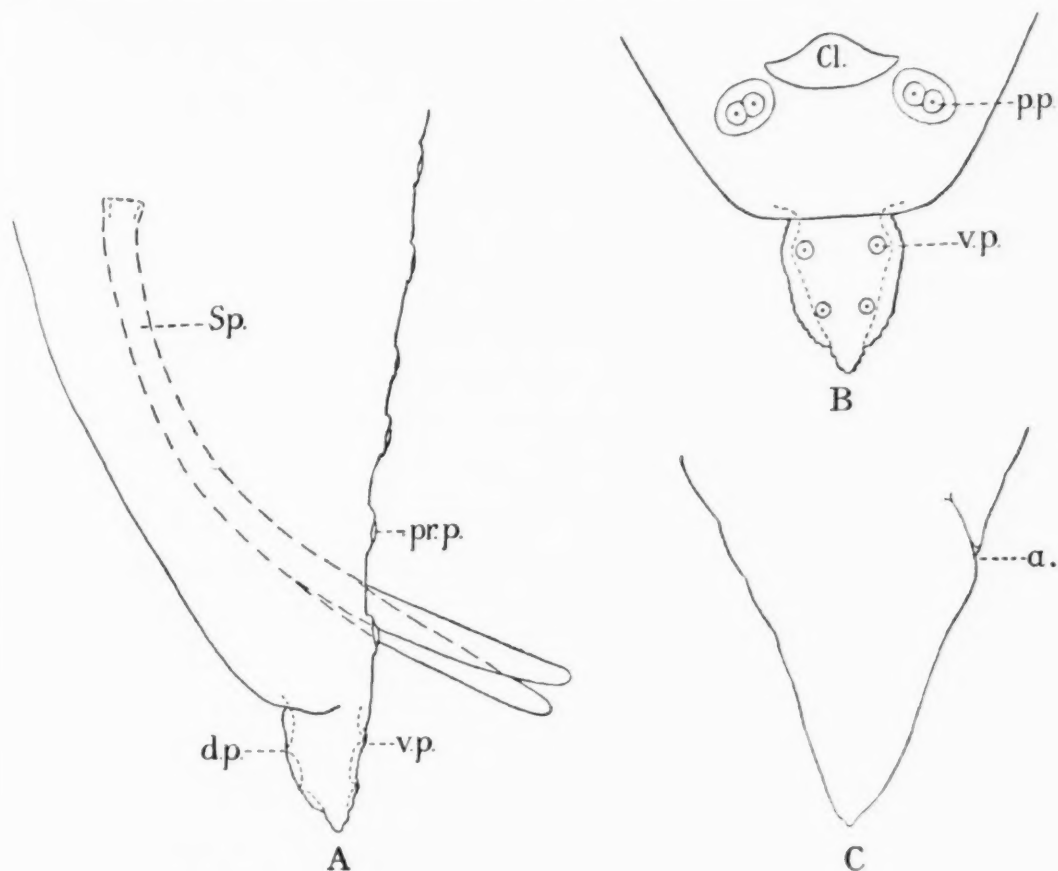


FIG. 2. *Ascaris* of Cattle. (A) Posterior extremity of male, lateral view,  $\times 75$ . (B) Posterior extremity of male, ventral view,  $\times 95$ . (C) Posterior extremity of female, lateral view,  $\times 40$ . *a.*—anus of female; *cl.* cloaca of male; *d.p.* and *v.p.*—dorsal and ventral papillae on appendix; *p.p.*—post-anal papilla; *pr.p.*—pre-anal papilla; *sp.*—spicule of male.

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## MOSQUITOES COLLECTED IN THE MANÁOS REGION OF THE AMAZON

BY

R. M. GORDON

AND

A. M. EVANS

*(Received for publication 11 August, 1922)*

### PLATE XIV

The culicidae recorded below were collected by one of us (R. M. G.) at Manáos Amazonas during 1921 and the beginning of 1922. A few of the species were taken in the town or its outskirts, but the great majority were obtained in the forests surrounding Macapa, a small saw-mill about fifteen miles from Manáos on the Rio Negro.

In this region only a dim light is present in the deeper parts of the forests. Here certain mosquitoes bite freely at all hours of the day, so a good deal of collecting was done by the party walking in single file, each individual 'bottling' mosquitoes as they lit on the person in front. Owing to lack of proper lighting facilities, little or no work could be done at night.

The breeding-places of these forest mosquitoes were difficult to locate, open pools are rare in the forest, and almost devoid of larvae when found. The most common breeding-places encountered were (1) reservoirs of water in natural crevices in the bark of trees; (2) rot-holes in trees; (3) water reservoirs in plants.

The food supply of these mosquitoes is doubtful, their chances of biting man are negligible, and animal and bird life seems extremely scarce.

Particular attention was paid to searching for Anophelines, none were discovered in the forest, the only ones recorded *Anopheles* (*Cellia*) *albimanus* being taken in the town or outskirts of Manáos.

Newstead and Thomas (1910) suggested that it was 'highly probable that other mosquitoes await the discoverer in a region so rich in insect life . . .'; among the present collection are many

species not recorded hitherto, of which four are new and two appear to be well marked varieties of existing species.

*Sabethes amazonicus*, sp. n.

**FEMALE. Head.** *Proboscis* long and slender, gradually enlarged apically. *Clypeus* and *tori* black with grey pruinosity. Scales of occiput with deep blue, violet and green reflections above, white beneath.

*Prothoracic lobes* covered with metallic scales with bright blue and green reflections varying according to the light; a row of coarse black bristles along the margin. *Mesonotum* largely denuded, the scales present similar to those of prothoracic lobes. *Scutellum* with lateral lobes metallic green scaled, mid lobe denuded. *Metanotum* with four coarse black setae. *Pleurae* and *coxae* with flat white scales.

*Abdomen*: Tergite of first segment with bright metallic green scales; white at sides. Scales of rest of tergites metallic with deep blue, pale blue, and green reflections according to the direction in which they are viewed. Sternites white scaled.

*Wings* with strong reddish-brown infuscation. Scales on knobs of halteres metallic yellowish-green.

*Legs* long and slender. Hind legs with paddles of long, outstanding scales involving distal half of tibia, metatarsus, and most of second tarsal segment; the longest scales about 1.2 mm. Front legs with tufts of outstanding scales on the distal half of the tibia and a few slightly raised scales at base of metatarsus; longest scales of tufts about 0.5 mm. Hind legs entirely without raised scales. Vestiture dark brown, with bronzy, coppery and violet reflections, femora, tibiae and metatarsi without white. Front tarsi with segments three, four and basal third of five white ventrally, segment four with dark spot at middle; mid tarsi with second, third and basal half of fourth segments white all round, except narrowly at the joints. Hind tarsi with segments three, four and five ventrally white, except narrowly at the joints.

Length: *c.* 7.0 mm. Wing: *c.* 5.5 mm.

*Type.* One female taken about three hundred yards deep in the forest, Macapa, 22nd December, 1921.

This species evidently comes very near *S. tarsopus*, D. & K., with which it agrees in having tufts of outstanding scales on the front and mid legs only. It differs from that species in the entire absence of white scales on the femora and tibiae and in the details of the tarsal markings.

*Sabethoides nitidus*, Theob.

Two larvae taken from a rot-hole in a 'Breau' (native name) tree in the forest near Manáos were brought to Liverpool alive. They were kept in an incubator at a temperature from 70° to 80° F., and one of them pupated, the pupa giving rise seven days later to a female *Sabethoides*. Although the specimen differs in certain details of coloration from Theobald's (1901), Howard, Dyar and Knab's (1915), and Dyar's (1919), descriptions of *S. nitidus*, it is referred to this species in the absence of male specimens from this region.

The specimen is more brightly coloured than typical *S. nitidus*, many of the head scales having brilliant pink and mauve tints, the scales of the prothoracic lobes and mesonotum are brilliant peacock-blue, not greenish-blue as in *S. nitidus*. The abdomen (fig. 1), seen



A.M.E.

FIG. 1. *Sabethoides nitidus*, Theo., from Manáos region, female abdomen from above.  
× c. 30.

from above, is coppery with violet reflections, and there are irregular basal patches of brassy scales on segments three and seven; broad, paired, dorsal patches, almost united in the middle, on segments four and five, and on segment six a complete broad, basal band. These brassy scales are quite conspicuous to the naked eye. Lateral basal white spots only present on last segment, not in all segments as in *S. nitidus*. The mid legs are white scaled above on the apical three-quarters of segment two and on segment three, four and five, except the extreme tip of five.

The larva of this species does not appear to have been described hitherto.

LARVA. Stage IV (fig. 2). Head broad. Mental plate with a

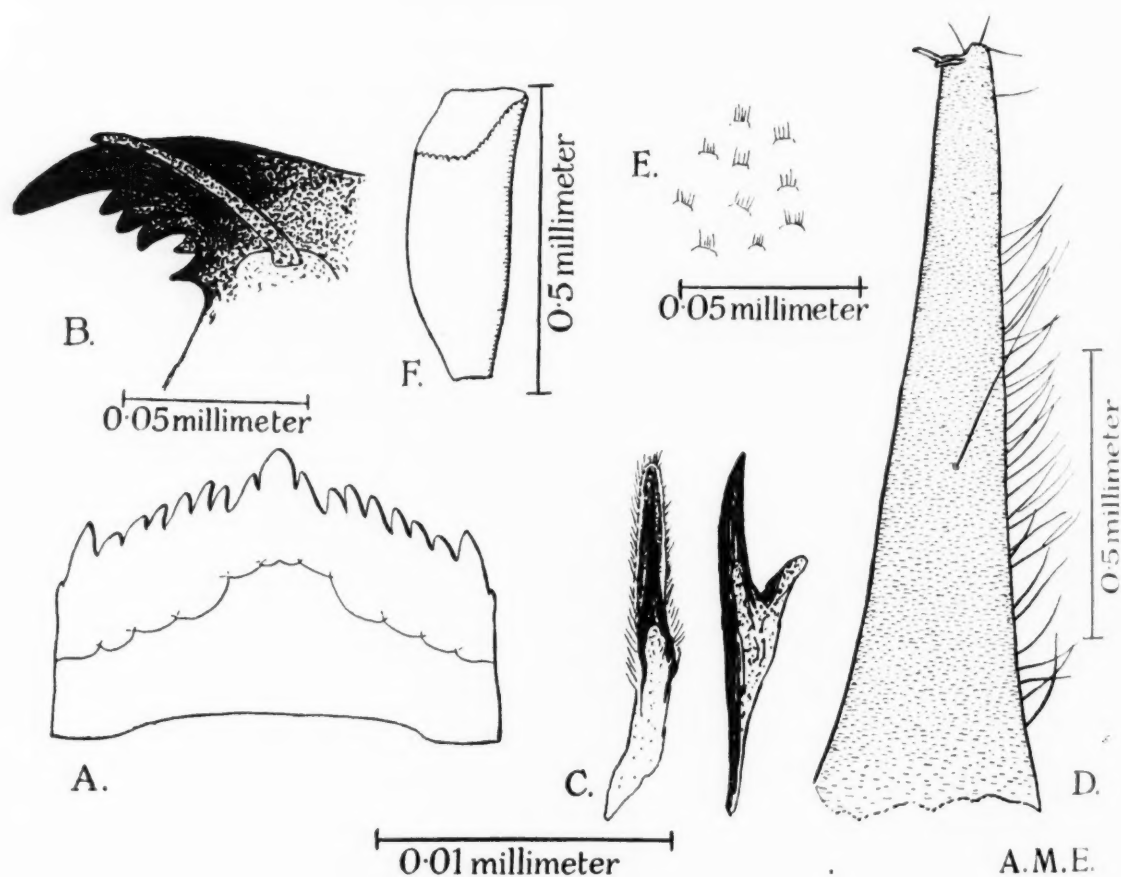


FIG. 2. *Sabethoides nitidus*, Theo., from Manáos region. A-E—larva; F—pupa. A—mental plate; B—dentition of mandible; C—spines of lateral comb (branched one in lateral view); D—siphon tube; E—part of surface of siphon tube enlarged; F—respiratory trumpet of pupa.

large median tooth and eight smaller ones on each side. Maxillae resembling those of *Sabethinus undosus* as described and figured by Howard, Dyar and Knab (1915), but, left maxilla with five teeth on



inner margin, right maxilla with four teeth in this position. Mandibles similar to *S. undosus*, but dentition (fig. 2 B), *six* (not four) teeth on a process, the terminal one large and falciform. Comb of eighth segment of twenty spines arising from membranous integument, spines thorn-shaped, some with a secondary pointed process (fig. 2 C). Siphon tube three and a third times as long as greatest width, surface with groups of microscopic hairs (fig. 2 E), a row of delicate sub-equal hairs arising from posterior margin for more than two-thirds of its length. Anal segment with plate reaching about half way down segment, dorsal angle on each side with two tufts of two setae; sub-ventrally a tuft of two and a tuft of three setae; lateral angles of plate with a tuft of two setae at each side. Anal gills sub-cylindrical, bluntly rounded, about three-fifths as long as siphon tube. Dorsal hooks of seventh segment, if present, so small as to be undetectable in crumpled pelt.

PUPA. Multiple tufts present on seventh and eighth segments. Respiratory trumpets moderately short and stout, opening wide (fig. 2 F). In life, abdomen with conspicuous dark segmental bands.

The coloration of this metallic scaled species appears to be extremely variable and open to a variety of interpretations. The extent of white on the hind tarsi is also subject to a considerable amount of variation in the descriptions of authors. The dorsal aspect of the abdomen was originally described by Theobald (1901) as 'deep metallic blue with basal coppery bands'; Howard, Dyar and Knab (1915) say 'dorsal vestiture metallic blue and green'; and Dyar (1919), in his coloration table, states that the abdomen has 'iridescent whitish, segmental bands.' A specimen labelled '*S. confusus*' in the British Museum was examined by one of us (A. M. E.), and the coloration of the abdomen above was found to be dark metallic violet with scattered pale scales, and on last segments pale basal bands.

Until a male from this locality is discovered, it must remain undecided whether the range of *S. nitidus* can be considered as extending as far westwards as Manáos, or whether the genus is here represented by a distinct species.

*Wyeomyia negrensis*, sp. n.

FEMALE. *Metanotum* with flat white scales and a few pale setae intermixed, a tuft of dark setae posteriorly. In other respects,

also, closely resembling *Cleobonnea occulta*, B. W. and B., except that scales on disc of mesonotum *broadly lanceolate*.

**MALE.** Coloration as in *C. occulta*, but legs differing considerably. Mid legs white ventrally and dark above throughout. Hind legs with femora, tibiae, metatarsi and basal quarter of second tarsal segment white ventrally; rest of tarsi brassy beneath.

**HYPOPYGIUM** (fig. 3). Side-pieces, tenth sternites and ninth tergites as in *C. occulta*. *Clasper* with a slender, recurved, basal 'lobe' (Dyar, 1919) (1), with retrorse pointed tip, and a wide dilation (*d.*), from which arise three lobes; an outer, rather slender lobe (2), with indications of a row of spines; a long curved lobe (3), with a row of teeth along inner side; an inner broad triangular

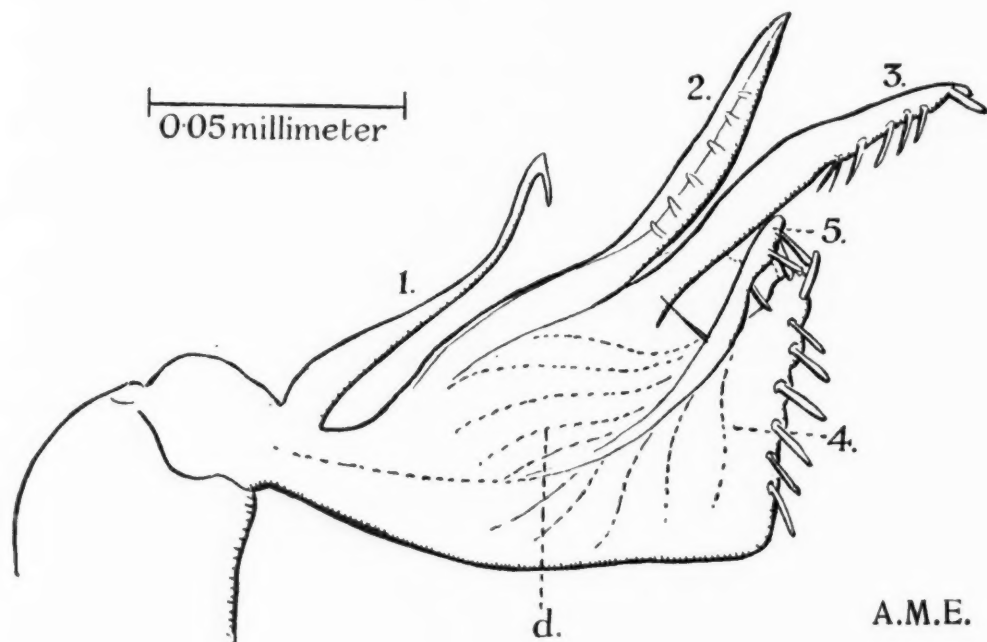


FIG. 3. *Wyemyia negrensis*, sp.n., male hypopygium, clasper. *d*—dilation; 1, 2, 3, 4, and 5, lobes of clasper.

lobe (4), with coarse teeth along distal edge; and a secondary lobe (5), with teeth on internal surface arising from fourth lobe.

**LARVA.** Stage IV. Head wider than long, widest at posterior angles. *Mental plate* triangular, with a median tooth and eleven sub-equal teeth on each side. Maxilla with a terminal transparent hook-like tooth, and a row of ten transparent teeth along inner side; a sub-apical tuft of delicate hairs, and near them a single seta on a tubercle, a row of hairs internally, and a short, stout spine near outer margin. *Thorax* with lateral dense tufts. Spines of comb of

eighth segment in a sub-triangular patch. Length of siphon tube about three times its greatest width, false pecten of four spines on distal half, three multiple tufts dorsally and a long multiple tuft ventrally at base. Anal segment with two pairs of dorsal tufts, one of five, one of two hairs; lateral hairs single, sub-ventral tufts of three and two hairs.

PUPA. A tuft of two long hairs bent as shewn in fig. 4 D near

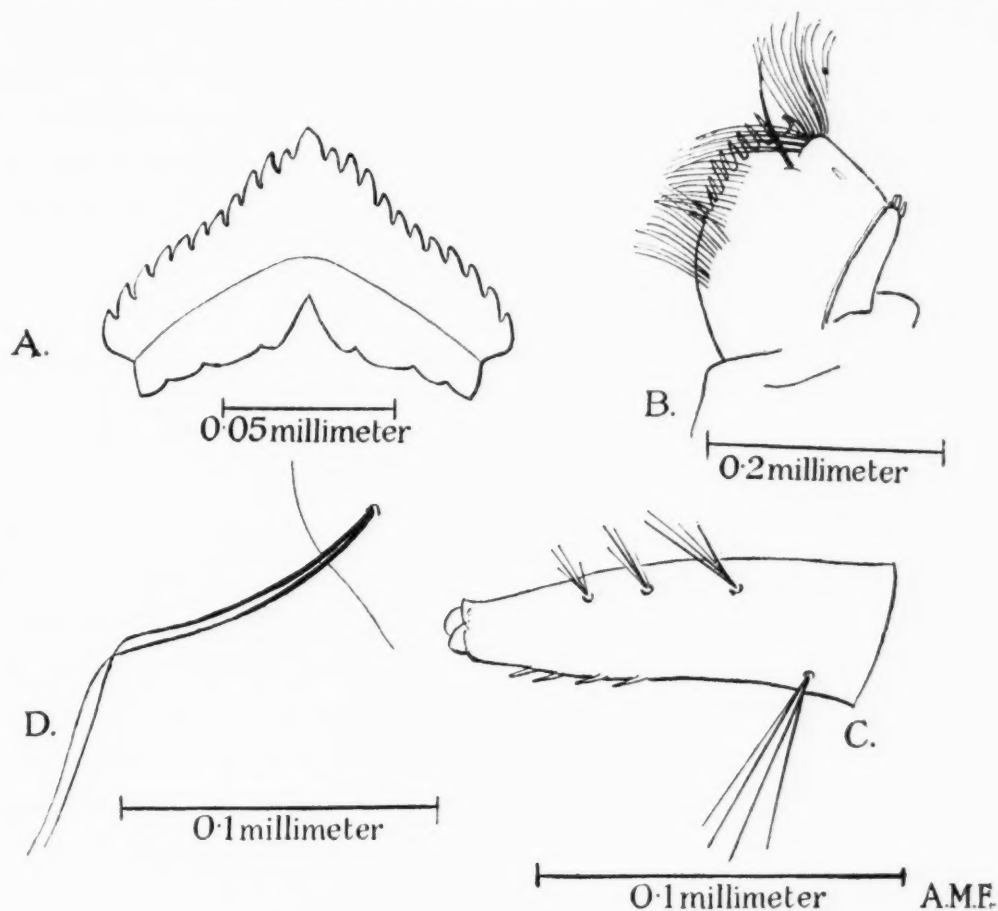


FIG. 4. *Wyeomyia negrensis*, sp.n. A, B, and C—larva; D—pupa. A—mental plate; B—maxilla; C—siphon tube; D—bent hairs of cephalothorax of pupa.

margin of each eye. A pair of sub-median tufts of eight branched hairs and sub-lateral tufts of four simple hairs behind insertions of antennae. Otherwise resembling pupa of *C. occulta*.

*Types*. One male and one female, bred from larvae living in the stem of *Bananeira braba* (wild banana) in the forest near Macapa, 20th Decemer, 1921. *Co-types*, five females from the same source.

This species is closely related to *Cleobonnea occulta*, B. W. and B., but there are marked differences in the coloration of the

legs of the male, and in the structure of the clasper. The mid legs of *C. occulta* are described as 'pale' beneath throughout, and the third, fourth and fifth segments white above. The hind legs are described as white beneath throughout, the last three tarsal segments brassy above. The male hypopygium has the clasper with only three lobes, closely resembling, 1, 3 and 4 of *W. negrensis* according to Dyar's (1919) figure of this structure; the lobes corresponding to 3 and 4 are differentiated much nearer the base than in *W. negrensis*; the dilation (*d.*) is absent. The branch (5) is evidently fused with the inner lobe (4) along its whole length in *C. occulta*. The quadrilobate condition of the clasper excludes *W. negrensis* from Dyar's sub-genus *Cleobonnea*, and it is here placed provisionally in the genus *Wyeomyia*.

*Culex (Neomelanoconion) chrysothorax*, Newstead and Thomas.

This species was frequently taken from a pool at the Bosque, about five miles from Manáos (Plate XIV, fig. 3).

Dyar (1918) suggested that it might be synonymous with *C. (Choeroporpa) chrysonotum*, D. and K.; and Bonne-Wepster and Bonne (1921), examined the types in the British Museum, and came to the conclusion that *C. chrysothorax* is a distinct species differing from *C. chrysonotum* 'by the broad white apices of its femora and tibiae,' and other characters. In view of the fact that most of the specimens in our series have the apices of the femora and tibiae only narrowly and faintly pale, the male hypopygium was examined and compared with that of *C. chrysonotum* described by Dyar (1920). Five specimens were examined; they showed quite distinct differences as follows:—

Inner branch of upper division of lobe of side-piece with larger appendage (fig. 5 A 1), a long, slender filament with recurved pointed tip, not 'somewhat flattened and blade-like' as in *C. chrysonotum*. Halves of mesosome (second plates, Dyar) (fig. 5 B) with a very long horn extending in the same direction as the basal hooks, arising nearer to the apex than the base; not near the base as in *C. chrysonotum*. It should be stated that, owing to the fact that the apical portions of the plates lie in a different plane from the basal main portion and from the horns, a considerable



number of totally different appearances of the whole structure may be obtained by altering the orientation (see fig. 5, C and D). In fig. 5 B the mesosome is drawn as seen when allowed to come to rest

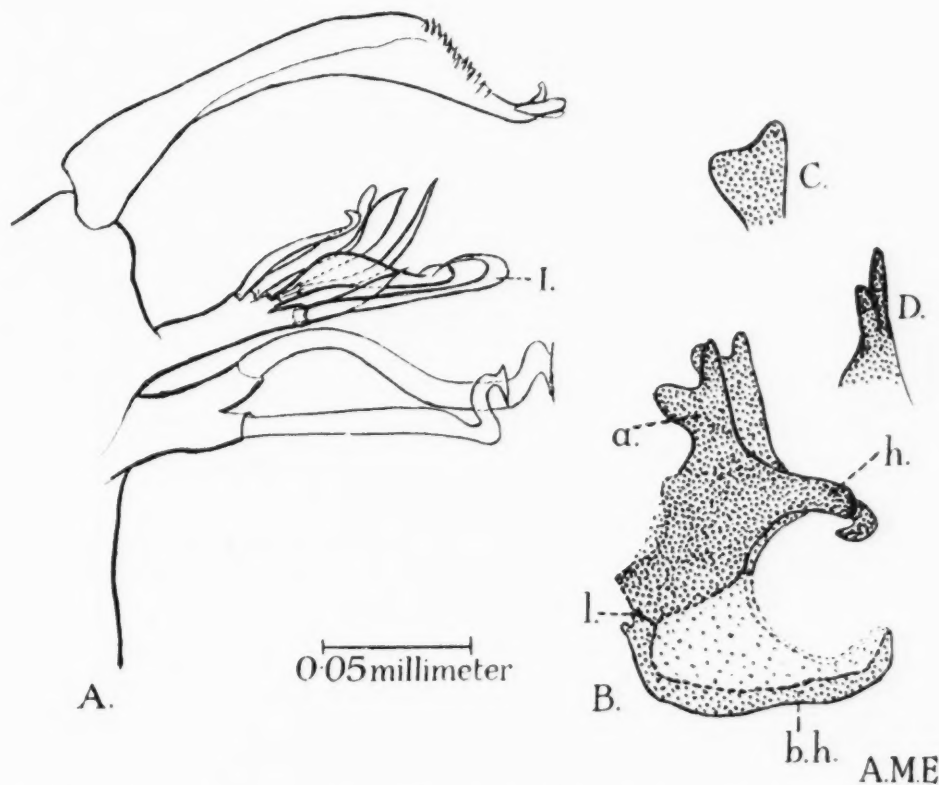


FIG. 5. *Culex chrysothorax*, Newstead and Thomas, male hypopygium. A—clasper and lobes of side piece, l—appendage referred to in text; B—entire mesosome, ventro-lateral view; b.h.—basal hooks; l—line of fracture between basal hooks and halves of mesosome; h—horn; C and D—ventral and lateral views of distal portion of half of mesosome.

on its side; as the two halves diverge at an angle, neither half is seen in true lateral view.

*Culex originator*, sp. n.

MALE. *Palpi* very short, slightly less than one-sixth of proboscis, slender, pointed. *Proboscis* swollen distally, bent beyond middle. *Occiput* with pale brown, narrow curved scales in middle, and whitish scales at sides and margins of eyes. Upright forked scales numerous, black. *Mesonotum*: integument dark grey, clothed with very narrow, curved, brown scales with slight greenish reflections, and numerous very long, coarse, black setae; two narrow, bare, dorsal stripes extending almost to ante-scutellar space, and a pair of wider, curved, sub-lateral bare lines extending from before wing roots outwards and backwards to lateral lobes of scutellum. Scutellum



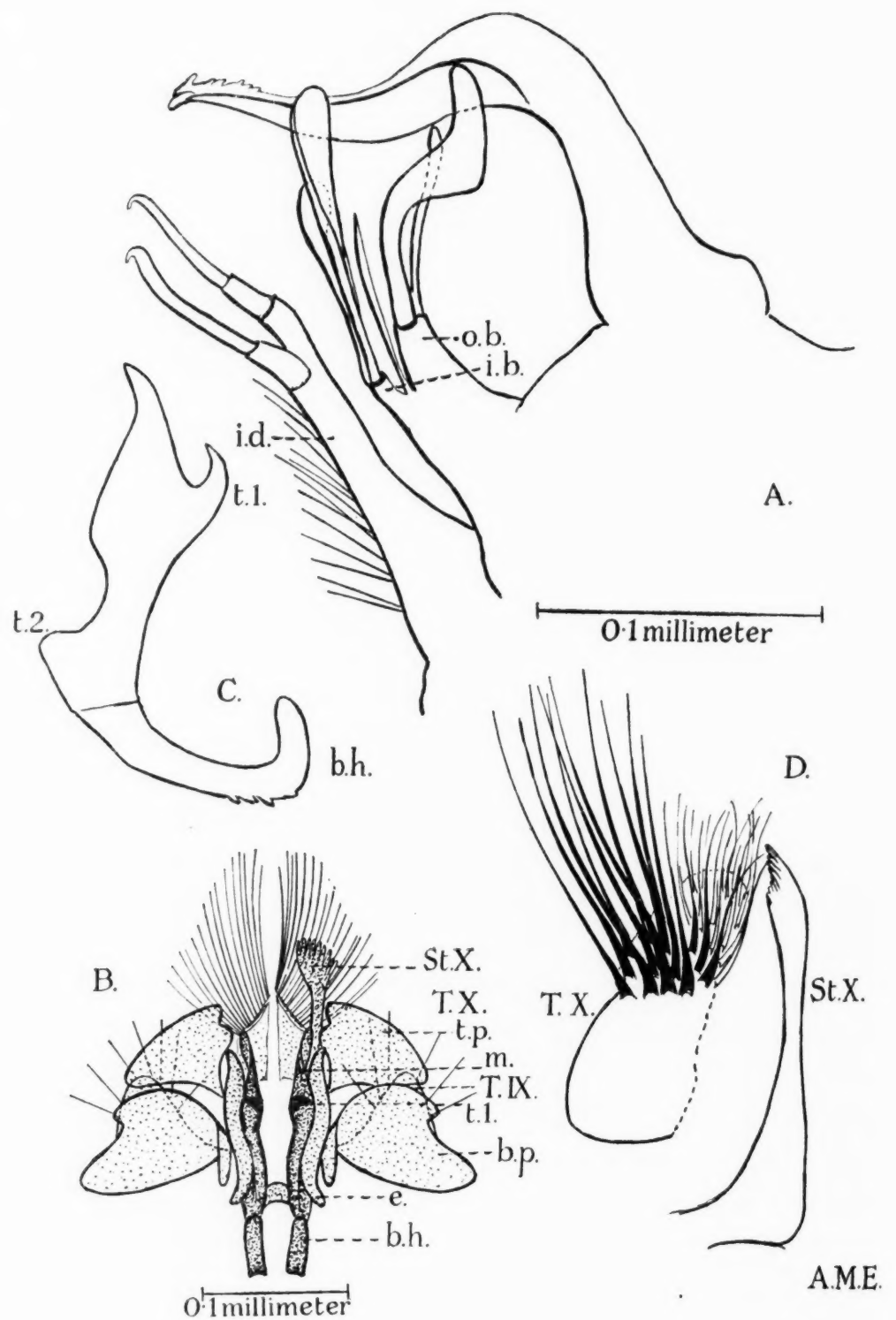


FIG. 6. *Culex originator*, sp.n., male hypopygium. *A*—clasper and lobes of side piece; *i.b.* and *o.b.*—inner and outer branches of outer division; *i.d.*—inner division; *B*—aedeagus, dorsi-ventral view; *b.b.*—basal hooks; *b.p.*—basal plate; *m.*—half of mesosome; *e.*—paramere; *St.X.*—tenth sternite; *T.IX.*—ninth tergite; *T.X.*—tenth tergite; *t.1.*—ventral tooth of mesosome; *t.p.*—transparent, triangular plate; *C*—half of mesosome and basal hook, lateral view; *b.b.*—basal hook; *t.2.*—dorsal tooth; *D*—tenth segment, lateral view. *A*, *C*, and *D* to same scale.

unicolorous with mesonotum. Pleurae green, with black setae and some pale ones. Abdomen with dark brown scales above, and on segments seven and eight very pale lateral basal spots. *Legs* clothed with dark brown scales; femora pale beneath; hind tibiae with a line of scales beneath with brilliant yellowish, silvery reflections, except on basal quarter and at distal extremity.

Wings as in *C. (Isostomyia) conservator*, D. and K.

Length: *c.* 3.5 mm. Wing: *c.* 2.5 mm.

**HYPOPYGIUM** (fig. 6) *Side-piece* short, rounded, width more than half the length. An area of dense setae near apex on inner side. *Clasper* angularly curved at right angles, gradually narrowing from bend to tip as shown in fig. 6A. Outer division of lobe of side-piece with distal half divided. Outer branch (*o. b.*) bearing a large filament distally expanded as shown in the figure, and a small spine. Inner branch (*i. b.*) of outer division of lobe of side-piece bearing a stout seta at base, and a pair of expanded filaments distally, one rather more distal than the other. Inner division of lobe of side-piece (*i. d.*) a stout arm, exceeding the outer division, with a row of setae arising from inner side and with two rod-like appendages with curved, pointed tips, the inner situated proximal to the outer. Tenth sternites with slender stem and expanded, comb-shaped apices, with nine teeth. *Apices of tenth tergites with a dense tuft of setae* (fig. 5D), the longest considerably longer than the tenth tergites. Halves of mesosome (second plate, Dyar), lateral aspect (fig. 5C) distally pointed, with a strong, pointed tooth on upper (true ventral) edge and a blunt tooth on lower edge near basal hooks; dorso-ventral aspect (fig. 5B, *m.*), distal portion spatulate. Basal hooks well developed, strongly curved. Ninth tergites (*t. ix.*) rounded, with four setae. 'Transparent triangular plates,' Dyar (*t. p.*), present between basal plates and ninth tergites.

**FEMALE.** Vestiture similar to the male, but upright forked scales of occiput dark brown; dorsal bare lines of mesonotum partially obliterated on posterior half; faint basal lateral, pale spots on abdominal segments three to six, and apical, lateral, pale spots on segment seven.

Length: *c.* 3.0 mm. Wing: *c.* 2.5 mm.

**LARVA.** Stage IV (fig. 7). Dorsal head hairs consisting of an inner pair of long tufts (*i. t.*) associated with a single long seta, and

an outer pair of shorter tufts (*o. t.*). Antennae normal, spiny. *Mental plate* (fig. 7 B) narrow with a very wide median tooth and eight smaller ones on each side, the last one remote. *Thorax* rounded, wider than long. *Siphon tube* (fig. 7 C) very long, length nearly eleven times the average width. Pecten not reaching beyond basal quarter, three long hairs beyond.

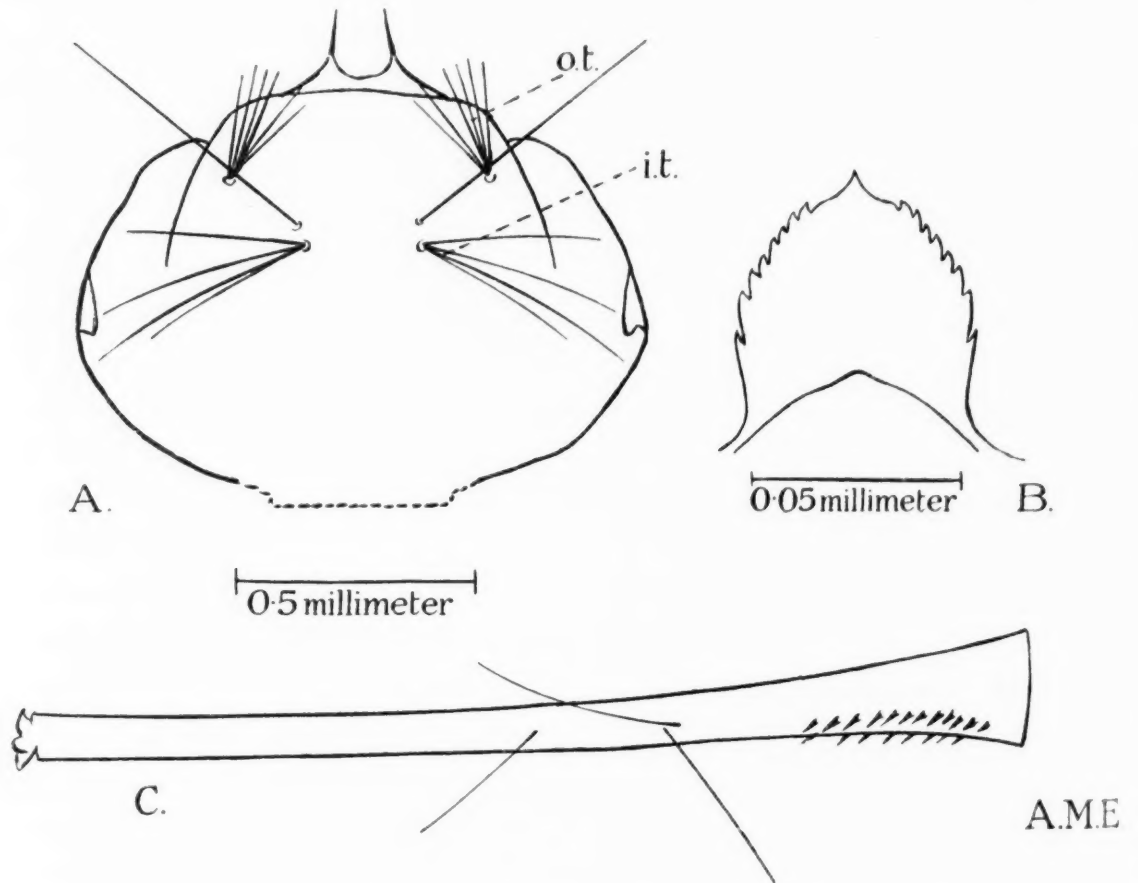


FIG. 7. *Culex originator*, sp.n., larva. A—head, dorsal view; B—mental plate; C—siphon tube.

*Type.* Male and female bred from larvae obtained from *natural* holes in the bark of the '*Carapana uba*' tree (native name = 'Home of the mosquito') about half a mile in the forest at Macapa, 21st December, 1921, emerged 1st January, 1922 (Plate XIV, fig. 2). *Co-types*, two males and two females from the same source, and one male from larva in rotten tree stump in forest at Macapa.

The characters of the male hypopygium readily separate this species from any other described species of *Culex*, but outwardly it closely resembles *Culex (Isostomyia) conservator*, D. and K. It differs from this species as described by Howard, Dyar and Knab

(1917) in having a line of brilliant yellowish, silvery scales beneath the hind tibiae, and faint pale segmental lateral abdominal spots. It would appear that the male of *C. conservator* has the upright forked scales of the occiput brown, not black, as in *C. originator*, and that the bare lines on the mesonotum do not extend more than half way back. Dyar (1922) discusses the hypopygial characters of *C. conservator* and the other two species of *Culex* with the male palpi as short as those of the female, *C. isostomyia bifoliata*, Dyar, and *C. micraedes corrigani*, D. and K. From his description of the shape of the clasper in *Isostomyia*, it seems probable that *C. originator* should be put in this sub-genus, although the divided outer division of the lobe of the side-piece, and the presence of conspicuous tufts of spines at the apices of the tenth tergites distinguish it markedly from the other two species. The latter character appears to be unique among American species of *Culex*.

*Culex corniger*, Theo.

A perfect female was taken in low herbage in a garden in Manáos, 7th June, 1921.

*Mansonia coticula*, Dyar and Knab.

Two females of this distinctive and beautiful species were caught about one mile deep in the forest at the saw mills, Macapa, 11 a.m. to 3 p.m., 7th December, 1921.

The type specimens were taken in the Panama region, and since its discovery the species does not appear to have been found elsewhere. Our specimens, however, agree with Howard, Dyar and Knab's (1915) description so exactly that we have no hesitation in assigning them to this species.

Females of *Manosnia titillans*, Walker, and *M. amazonensis*, Theo., were frequently taken biting man by day in the forest near Macapa.

*Haemagogus (Stegoconops) equinus*, Theob.

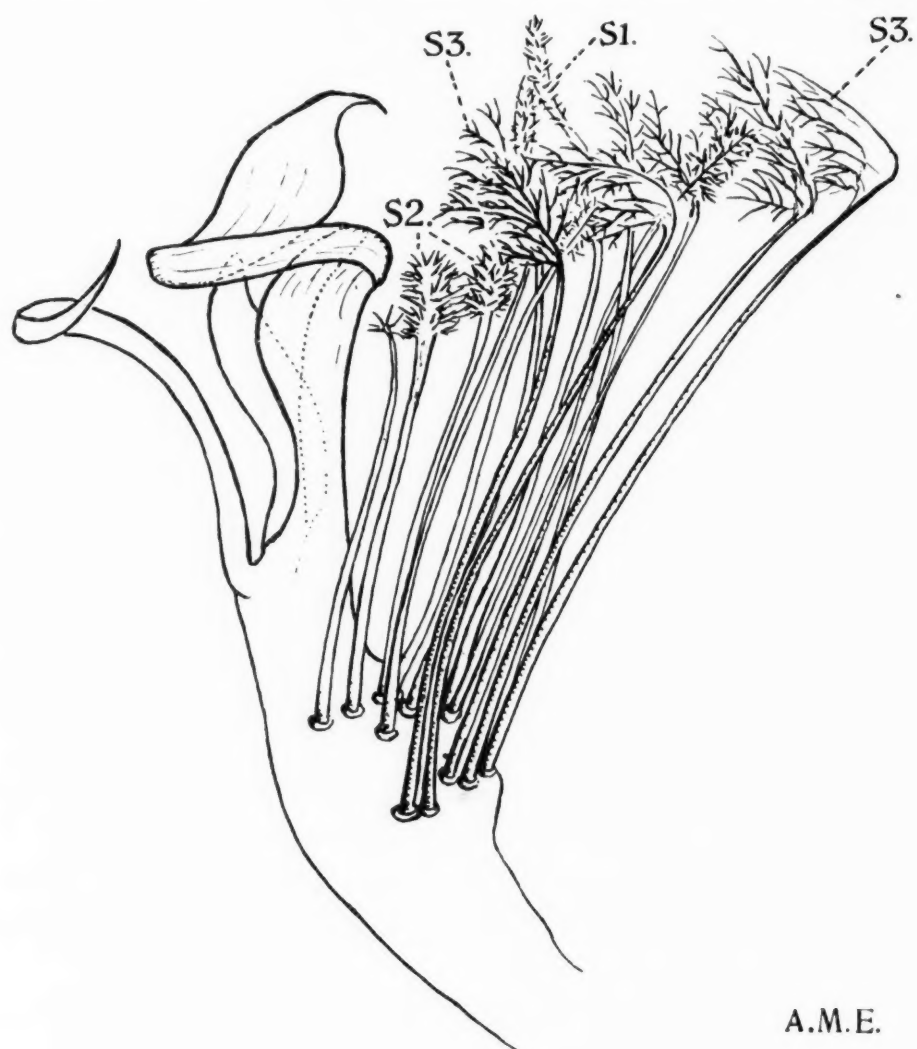
Three females taken in the forest near the saw mills, Macapa, 11 a.m. to 3 p.m., 7th, 22nd and 23rd November, 1921, are referred to this species.

*H. equinus* has not hitherto been recorded from the Manáos region, but it has a very wide distribution in South America, Dyar (1921), and in the absence of males the present specimens must be regarded as this species.

*Psorophora lutzii*, Theo.

In addition to numerous females, a male of this species was caught in the forest near Macapa saw mills, 10 December, 1921.

The male of *P. lutzii* does not appear to have been described hitherto. It differs from the female in having yellow scales immediately in front of the ante-scutellar space, a character which



0.1 millimeter

FIG. 8. *Psorophora lutzii*, Theo., male hypopygium, apex of claspette (harpagone); S.1, S.2, S.3 types of setae referred to in the text.

was confirmed by Mr. F. W. Edwards, who kindly examined the male specimens in the British Museum collection.

**HYPOPYGIUM** (fig. 8) with side-pieces, tenth sternites and aedoeagus as in *P. posticus*. The claspettes (harpagones) H., D.



and K. (1917), apically expanded on inner side; with a large terminal 'S'-shaped leaf, a much smaller curved leaf, and a narrow pointed filament distally curled; internal surface with fourteen (this number may be subject to slight variation) long setae with expanded apices. The setae of three types:—I. (fig. 8, s. 1) with apices slightly swollen, bearing short simple hairs; II. (s. 2) apices considerably expanded, with longer, very delicate hairs, some of which branched; III. (s. 3) apices produced into large membranous expanses, with fine, filamentous, branched processes.

Females of this species and of *P. posticatus*, Wied., were the commonest mosquitoes biting by day in the forest near Macapa.

*Aedes (Finlaya) oswaldi*, Lutz, var. *braziliensis*, n. var.

Two perfect males of the *Finlaya* group of *Aedes* were referred to this species, although they differed from it in certain respects.

The differences are tabulated below:—

	<i>A. oswaldi</i>	<i>A. oswaldi</i> var. <i>braziliensis</i>
Mid legs ... ..	2nd tarsal segments with basal third white	2nd tarsal segment with basal half white.
Hind legs ... ..	Metatarsus with apical quarter; 2nd tarsal segment with basal third white	Very narrow white rings at these places
Segment VIII of abdomen ...	Dorsally silver scaled	Dorsally dark scaled

The anterior three-fifths of the mesonotum are covered with very thick, bluish silvery, narrow curved scales, the whitish area being deeply incised behind. The hypopygium resembles that of *A. oswaldi*, but the clasper is capitate distally, not pointed as in Howard, Dyar and Knab's (1912) figure of that species.

*Type* and *co-type* males bred from larvae found in hollow in tree stump, about one and a half miles deep in forest at Macapa, 8th December, 1921.

*Megarhinus horei*, sp. n.

MALE. *Proboscis* about nine-tenths of the length of the wing, slender, tapering to a point; *palpi* slightly longer than proboscis, vestiture of all but last segment above predominantly peacock-blue, violet towards ends of segment and in front of false articulation; scales at dilated articulations and false articulations white, with pale mauve reflections; all but last segment with pale scales, appearing brassy or whitish according to the direction of the light. Last segment bronzy scaled with deep purple reflections. *Antennae* with hairs of whorls blackish-brown, second segment dotted on distal two-thirds of inner side, with metallic scales appearing peacock-blue, purple or whitish in different lights; tori black with silvery pruinosity. *Clypeus* short, ochraceous brown, darker in centre, with whitish pruinosity. *Occiput* mostly covered with olivaceous green scales, pale blue ones in front and at sides, white scales along ocular margins and beneath. *Prothoracic lobes* with brilliant blue scales above, violet ones towards margin, and white scales beneath, a row of coarse black setae along margin. *Mesonotum*, viewed without magnification from above, bronze, with a median peacock-blue stripe about one-fifth of the width of the mesonotum at the middle, posteriorly the blue area widens and coalesces with blue patches over the roots of the wings; bronze area bordered by whitish blue at edges of disc. Magnified about fifty times with binocular microscope, the bronze area seen to consist of spindle-shaped scales with brassy, coppery, greenish or light blue reflections, according to the direction in which they are viewed; the scales directed outwards on anterior, inwards on posterior half; blue area consisting of broad, flat, backwardly directed scales, bronze when viewed from behind, metallic peacock-blue from above; pale scales bordering disc at sides and in front broader than spindle-shaped scales on disc, very transparent, whitish, with azure-blue and pale greenish-blue reflections. Scales forming patches over roots of wings peacock-blue, with lighter blue and greenish reflections. *Scutellum*, without magnification bright metallic blue, very slightly paler than blue of mesonotum; with magnification fifty times, scales on mid and lateral lobes similar, appearing peacock-blue with deep violet reflections, pale blue, translucent pale green or translucent brassy, according to

the direction of the light; mid and lateral lobes with groups of stout, black setae. Pleurae and coxae with patches of dense creamy-white scales. Spiracular bristles seven, black; pre-alars seven, pale straw coloured; upper mesepimerals numerous, very pale.

*Abdomen* above, with segment one metallic pale blue, segments two, three and four with peacock-blue scales, remaining segments and side-pieces bronzy brown, with violet reflections. Sides of segments with apical patches of creamy scales, brilliant blue scales at base, some of scales with whitish and mauve reflections in certain lights.

Scales of venter creamy with silvery reflections, a median dark stripe of bronzy scales with peacock-blue reflections, lateral ciliation short, delicate, pale yellow.

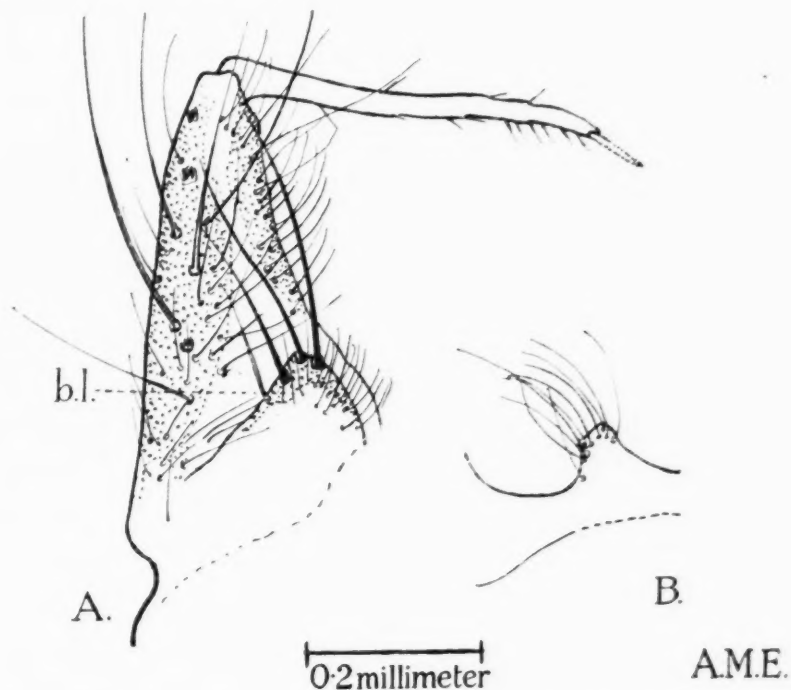


FIG. 9. *Megarbinus borei*, sp.n., male hypopygium. A—side piece; b.l.—basal lobe; B—ninth tergite.

*Legs.* Vestiture of dark scales with deep blue and purple reflections. Femora brassy beneath, knees entirely dark. Hind tarsi with fourth segment white, except at base and apex, and a very narrow line of dark scales on upper surface behind.

**HYPOPYGIUM** (fig. 9). Basal lobe of side-piece with three stout setae at apex, of which two very long, reaching almost to insertion of clasper. Ninth tergites short, with about eleven fine setae.

Length: *c.* 10 mm. Wing: 7 mm.

FEMALE. *Palpi*: coloration above similar to male, but scales at articulations dark, with paler violet reflections; brassy scales at sides confined to basal third, rest with reddish-purple reflections.

*Mesonotum* entirely covered on disc, except on posterior extremity, with dark bronze spindle-shaped scales, which appear deep blue with purple reflections when viewed in a direction parallel to their long axis; posterior portion between wing roots with flat scales of similar coloration. In the normal position the thorax appearing bronzy-brown, except at posterior extremity, and in irregular patches on middle regions of posterior half which appear deep ultramarine blue, owing to the antero-posterior direction of most of the scales in these regions. Laterally the scales directed more or less at right angles to the longitudinal axis, and, therefore, only appearing blue when the thorax is viewed from the side.

*Abdomen*. Similar to male, but blue colour deep ultramarine, and on last two segments above an almost complete apical fringe of brassy scales.

*Legs*. Similar to male, but mid legs with segments two and three white on anterior and dorsal surface, except narrowly at apices and bases; hind legs with segment four entirely whitish scaled, segment five with whitish scales on basal two-thirds anteriorly.

LARVA. Stage IV (fig. 10). *Head*, sub-quadrate, about as wide as long, insertions of antennae rather prominent, front margin deeply emarginate, produced into large prominent lobes on each side, bearing mouth brushes. Antennae cylindrical, slender, rather long, smooth, hairs sparse, internally a tuft of two hairs, externally two longer hairs on apical fourth; apex with a jointed and an unjointed appendage and a hair. Dorsal head hairs fine, three on each side behind frontal lobes, behind and internal to antennae a row of three on each side and a minute tuft internally; a single hair internal to eyes and a small branched one apparently rising from eyes. Mouth brushes consisting of nine curved blades. Labial structures (fig. 10, E, F, G) consisting of a broad chitinous fold (sub-mentum ?) hairy in middle distally with internal surface with median area heavily chitinised, tuberculate and a large stout tooth (*m. t.*) arising in centre: a mental plate attached to dorsal surface of fold (see fig. 10 G, which shows relative position of parts of labium), having a very shallow median tooth with a small tooth on each side and



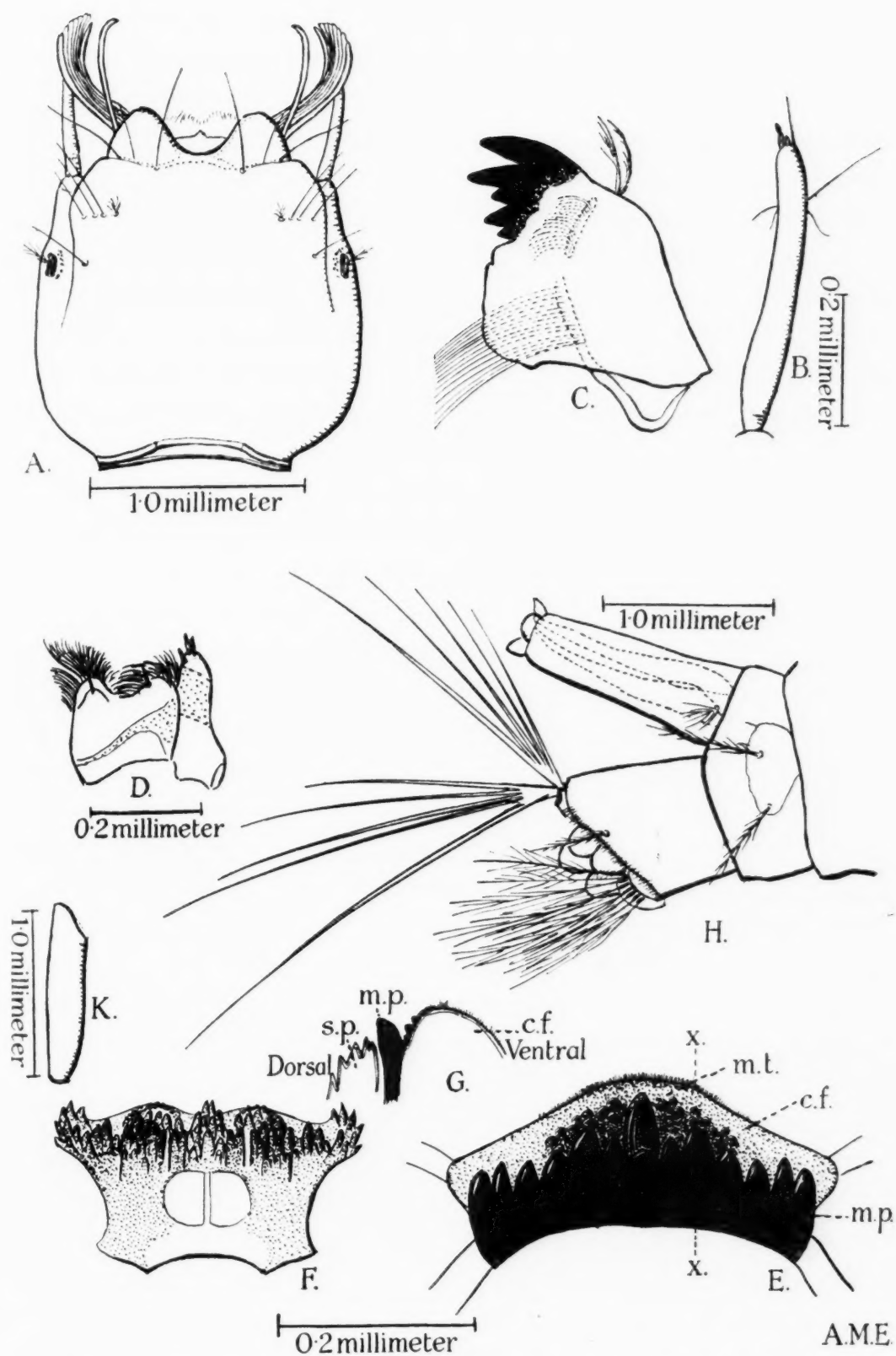


FIG. 10. *Megarbinus borei*, sp.n. A-H—larva. K—pupa. A—head, dorsal view; B—antenna; C—mandible, to same scale as D; D—maxilla; E—labium, ventral portion; c.f.—chitinous fold; m.p.—mental plate; m.t.—median tooth of chitinous fold; F—secondary plate of labium; G—sagittal section of labium at x-x, s.p.—secondary plate; H—segments VIII and IX; K—respiratory trumpet of pupa.



five large ones beyond on each side; and a "secondary plate" of the form shown in the figure, with the distal portion thickly dentate on dorsal surface, and the teeth tending to form a median, two lateral and intermediate groups. *Mandible* with a pair of sparsely feathered hairs (*h.*) on outer side; dentition of five teeth of which two very large, ensiform; dorsal surface with a row of short fine hairs, and a proximal row of long hairs. *Maxilla* rectangular, bi-lobed distally, edges of lobes densely setose, inner lobe with a stout spine on a prominence behind insertions of hairs; outer lobe with a short, stout sensory spine rising from a tubercle almost at edge, palpi with a chitinous plate as shown in fig. 10 D, and three rudimentary jointed digits. *Thorax* rounded, the stout hairs spinulose. *Abdomen*: lateral tufts of hairs not arising from large chitinous tubercles. Siphon tube about two and a half times as long as wide, no pecten, a single tuft near base. Large plate on side of eighth segment with two stout spinulose hairs on its posterior margin. Anal segment about as long as wide, ringed by the plate; dorsal tufts of two long brushes on each side, a single spinulose lateral hair. Anal gills very short, bud-shaped.

PUPA. Respiratory trumpets as shown in figure.

Length: *c.* 13 mm.

*Types.* One male and one female, bred from larvae found in stems of *Bananeira braba* (wild banana) in the forest near Macapa, 21st December, 1921. The species is dedicated to Mr. A. T. S. Hore in recognition of valuable services, which he rendered during the collecting expeditions that were undertaken.

BIONOMICS. The larvae of this mosquito were first discovered together with those of *Wyeomyia negrensis*, sp. n., in a stretch of forest about four miles from Macapa. As mosquitoes were extremely plentiful at this point, a small tract of forest was carefully searched for breeding-places, the larvae referred to were found by splitting up the fronds at the base of a '*Bananeira braba*' (wild banana tree). As we were shifting camp the same day, the larvae had to be transported some distance in a hot sun, and none of them survived the journey.

A few days later a wild banana (Plate XIV, fig. 1) was selected growing at the edge of the forest about ten miles from the spot previously examined, this was cut down close to the roots and transported to camp, where it was placed in a petrol tin, the outer

fronds torn off, and finally the base split up with knives. No larvae were found till the base of the tree was reached, those found were lying in the innermost fronds fully six inches from the outer circumference of the tree. The larvae were found to be carnivorous and had to be kept in separate tubes, where they were fed on a diet of *Culex quinquefasciatus* (*fatigans*) larvae and pupae, of which they readily destroyed two a day. In captivity they spent most of their time at the bottom of the jars, only coming to the surface at long intervals.

No eggs were discovered, so the length of larval life is unknown. The average pupation period was found to be six days.

*Uranotaenia calosomata* var. *albitarsis*, n. var.

The specimens agree with typical *U. calosomata*, D. and K., in the coloration of the head, thorax and abdomen, but the front and mid tarsi have the last three segments creamy-white scaled, not as in *U. calosomata*, in which they are described as having 'a brassy lustre particularly apically.' Hind tibiae with a conspicuous bluish-white stripe extending the whole length behind; in *U. calosomata* the hind tibiae have only the tips narrowly silvery white. Proboscis with a bluish-white line on basal four-fifths beneath, apparently absent in *U. calosomata*.

HYPOPYGIUM with spines on basal lobe of side-piece extending beyond the apices of the side-pieces; they are very short in Howard, Dyar and Knab's (1912) figure of the hypopygium of *U. calosomata*.

*Type.* Male and female bred from larvae taken in old iron bath at the saw mills near Macapa, 20th January, 1922; *co-type*, female from the same source.

Other species of *Uranotaenia* taken were *U. geometrica*, Theo., ♂ 1, flying in low herbage, Manáos, October, 1921; and *U. lowii*, ♀ 1, Manáos, 15th January, 1922.

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## EXPLANATION OF PLATE XIV.

- Fig. 1. Wild Banana (after having been cut down). Breeding-place of *Megarhinus hoeri*, sp. n. and *Wyeomyia negrensis*, sp. n.
- Fig. 2. 'Carapana Uba' Tree. Breeding-place of *Culex originator*, sp. n.
- Fig. 3. Breeding-place of *Culex* (*Neomelanoconion*) *chrysothorax* at Boski, Manáos.





FIG. 1



FIG. 2



FIG 3.



# TRYPANOSOMA RHODESIENSE IN A CASE OF SLEEPING SICKNESS FROM THE SUDAN

BY

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DIRECTOR OF THE WELLCOME TROPICAL RESEARCH LABORATORIES, KHARTOUM

*(Received for publication 25 September, 1922)*

Three cases of human trypanosomiasis were recently brought to Khartoum by Captain Mackinnon, M.C., R.A.M.C., Medical Officer in Charge of the Sleeping Sickness Camp at Tembura, in the Bahr-el-Ghazal Province of the Sudan.

Gland puncture carried out two months previously had proved positive for trypanosomes in all three cases; in order, however, to minimize the possibility of spreading infection during their journey through fly-infested areas, each patient had received two injections of 0.5 gramme atoxyl.

On arrival in Khartoum gland puncture was again carried out, but trypanosomes could not be found in the several preparations examined; it was decided, however, to inoculate animals with the gland juice obtained from one of the patients. The case selected showed evidence of somnolence with a well marked enlargement of the lymphatic glands of the neck and axilla, as well as a slight degree of pyrexia. An emulsion of the gland juice with a sterile 1 per cent. solution of sodium citrate was prepared, and inoculated subcutaneously into three healthy gerbil rats.

At the end of sixty-six days one of these rats showed an intense infection with trypanosomes in its peripheral blood; stained preparations demonstrated the presence of posterior nucleated forms.

Further details regarding this trypanosome and its pathogenicity for various animals will be published later by Captain Whitehead, M.C., R.A.M.C., Government Bacteriologist; suffice it to say that its morphological characters and pathogenicity for animals, justify

the conclusion that the trypanosome is *T. rhodesiense*, an opinion shared by Professor Warrington Yorke, who kindly examined stained blood films from infected rats, as well as other data submitted.

Investigations regarding the insect carrier of this trypanosome remain to be carried out; it is of interest, however, to note that *Glossina fuscipes* and *G. morsitans* are ubiquitous in the district of Tembura.

The writer is indebted to the Principal Medical Officer, Egyptian Army, for facilities granted in obtaining the material which forms the subject of this brief paper.







For the Treatment of  
 Schistosomiasis  
 Trichuriasis  
 Leishmaniasis  
 (KALA-azar and  
 Oriental Sores)

TRADE  
 MARK

# ANTIMONY TARTARATE

Contains 10% of Antimony Tartarate. Each  
 of 10 Sodium Chloride Tablets. Each  
 Sodium Chloride Tablet contains 0.1 g.  
 Dissolve in 100 cc. of water. Each  
 strength of solution is 10 mg. of Antimony  
 Tartarate per 100 cc. of solution. Sodium  
 Chloride is added to the solution to  
 Antimony Tartarate. The solution of the  
 solution is 10 mg. of Antimony Tartarate per  
 100 cc. of solution.



Tablet (Antimony)

Label

BURROUGHS WELLS

No. 1

(Ant.)

No. 2

(Ant.)

quest

ON

## CORRIGENDA

- P. 133. 4th line from foot for *TETRAPHYLLIDEA* read  
*TETRAPHYLLIDAE*.
- P. 151. Line 17, for *PROTEOCEPHALALIDAE* read  
*PROTEOCEPHALIDAE*.
- P. 160. Legend beneath fig. 1, and 3rd line from foot, for  
*A. costalis*, Theo., read *A. costalis*, Loew.
- P. 161. Legend beneath fig. 2, for *A. costalis*, Theo., read  
*A. costalis*, Loew.
- P. 162. Legend beneath fig. 3, for *A. costalis*, Theo., read  
*A. costalis*, Loew.
- P. 173. Legends beneath figs. 11 and 12, for *Aedomyia africanus*  
read *Aedomyia africana*.
- P. 327. Line 28, for *Manosnia* read *Mansonia*.

For the Treatment of  
 Schistosomiasis  
 in Males  
 Leishmaniasis  
 KALA-AZAR  
 ORIENTAL S

ANTI

Constitute a solution of 1.53d. with  
 of Sodium Chloride  
 Dissolved in each  
 strength of normal  
 saline solution of Sodium  
 Antimony  
 The dose of this  
 solution is 10 cc.



Antimony

BURROUGHS-WELLS

## AN UNUSUAL TYPE OF NODULAR LEPROSY IN THE SUDAN

BY

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(DIRECTOR, WELLCOME TROPICAL RESEARCH LABORATORIES, KHARTOUM)

*(Received for publication 20 August, 1922)*

### PLATE XV

Leprosy has a wide distribution in the Sudan, but is by no means the common entity among the native population as in the countries of the Far East. Of the varieties observed, the nodular or tubercular type is apparently the more common, presenting little difficulty in clinical diagnosis, and occurring usually in the form of well-developed nodular or tubercular lesions of the skin and tissues, as illustrated in a recent paper published by the writer.

The case, which forms the subject of this paper, differed clinically from the usual type of leprosy observed in the Sudan, and appears worthy of record, inasmuch as it presented certain features which certainly obscured the diagnosis.

The patient was an Egyptian, about 30 years of age, who stated he had suffered from an eruption of the skin for a period of one year. According to his history, the eruption apparently commenced on the face in the form of small shotty papules, similar ones eventually appearing on the forehead, ears, trunk and upper and lower extremities. The eruption caused little or no inconvenience, but as it appeared to be getting more extensive and causing some disfigurement, he sought medical advice. His previous medical history contained little of interest. There was no history of syphilis; the patient, however, admitted that his wife had an abortion a few months previously. The case having presented certain clinical features akin to a syphiloderma, and as facilities for proving this by laboratory examination were lacking, he was treated



with a course of injections of '606,' but failed to show any improvement; in fact, his condition became progressively worse.

When seen by the writer, the patient was well nourished and in fair general health. On examination, it was found that the skin of the face, neck, anterior and dorsal aspects of the trunk, and the flexor and extensor aspects of the arms and legs showed numerous miliary papules varying from 0.3 to 0.5 centimetres in diameter. The majority of these were discrete, with a smooth surface, circular contour, pink colour, and of a shotty consistency; some of them showed a slight inflammatory reaction at the base. In certain areas, more especially on the neck and arms, many of the papules showed a circular depression or umbilication in the centre, while others showed simply a pale central area (Plate XV, fig. 1). No pustulation was noted. The largest were on the face, and here the majority of them were discrete, whereas those on the ears had coalesced and caused considerable thickening of the tissues, producing an appearance not unlike that of *haematoma auris*. Papules were also present over both superciliary regions, where a slight degree of madarosis was noted. The skin of the arms was more affected than that of the lower extremities, both flexor and extensor aspects being involved. The intervening portions of the skin presented no abnormalities, except in a few areas on the face where there was a certain degree of erythema.

No nodules or ulcers were detected in the buccal mucous membrane; but the posterior fauces and larynx were slightly inflamed, which accounted for the somewhat hoarse voice of the patient. The submaxillary and axillary lymphatic glands were slightly enlarged and firm on palpation.

No abnormalities were detected in the heart, lungs, liver and spleen. The patient's temperature at the time of examination was normal, but he admitted that he suffered occasionally from attacks of pyrexia.

Two of the shotty papules from the arm were excised, fixed in picric alcohol, and embedded for sections. Microscopical examination of haematoxylin-eosin stained preparations showed the cytological changes associated with a granuloma, and special staining methods demonstrated the presence in the tissues of large numbers of acid-fast bacilli, morphologically resembling leprosy bacilli



(Plate XV, fig. 4). These were especially well seen in sections stained by carbol-fuchsin, decolourized in 10 per cent. sodium sulphite, and finally counterstained with an aqueous solution of methylene blue containing 1 per cent. sodium carbonate.

#### *Histopathology of a nodule*

Sections showed a thinning not only of the horny layer of the epidermis, but also of the rete mucosum, the cells of the latter consisting chiefly of oval and columnar cells (Plate XV, figs. 2 and 3). Beneath the lower border of the rete mucosum there was a narrow zone, poor in cellular elements, which stained feebly with tissue stains (fig. 3). Special staining reagents showed it was composed of fibrous tissue, which apparently had undergone a hyaline or vitreous degeneration. Beneath this narrow zone there was a marked cellular reaction in the upper part of the corium. The cells here were composed chiefly of plasma and lymphoidal cells (fig. 2); but no giant cells were present. This cellular infiltration occurred also to a less degree in the pars reticularis, but it varied in intensity in different areas of the nodules. Where well marked, it encroached on the narrow or vitreous zone, extending almost to the rete mucosum (fig. 2). Where it was slight or hardly present the vitreous zone was wider, and beneath it the corium appeared to be composed of loose, oedematous-looking connective tissue in which dilated lymphatic vessels filled with lepra bacilli were noted (fig. 4). This area was rich in lepra bacilli, dense masses extending throughout the *pars reticularis* down to the subcutaneous tissues; they were not found either in the sebaceous glands or in the hair follicles; indeed, these structures, like the blood vessels, appeared to be unaffected. In the vitreous zone, beneath the rete mucosum, only a few single bacilli were found; none were located in the rete or in the horny layer. The infected area appeared to be confined to the corium, the infection reaching that portion of the skin via the lymphatics.

#### *Remarks on the Case*

In considering the condition from a clinical aspect, it must be admitted that the case presented certain puzzling features.

The discrete nature of the eruption and the umbilication of some

of the nodules, their size and extensive distribution, together with the clinical history of only twelve months' duration, compelled one to consider and eliminate various skin eruptions that have been studied in this country. Of these may be mentioned *Molluscum contagiosum*, generalised vaccinia, Lichen hyperkeratosis, cutaneous Leishmaniasis, prurigo, yaws and syphiloderma. Most of these could be readily differentiated; the possibility of the case being one of leprosy, occurring, moreover, in a Government official, did not occur to the writer, nor was it suspected by the various medical men who examined the case.

It was left to the histological examination of the excised nodules to throw light on the nature of a condition which might well be termed miliary leprosy.



## EXPLANATION OF PLATE XV

- Fig. 1. Illustrating the eruption.
- Fig. 2. Photo-micrograph of a section of a papule, showing the cellular infiltration encroaching on the rete malpighii.  $\times 190$ .
- Fig. 3. Photo-micrograph of a section of a papule, showing the narrow hyaline zone of degeneration subjacent to the rete malpighii.  $\times 190$ .
- Fig. 4. Photo-micrograph of a section of the corium stained with carbol-fuchsin methylene blue. The dark stained areas represent clumps of lepra bacilli.  $\times 800$ .



FIG. 1

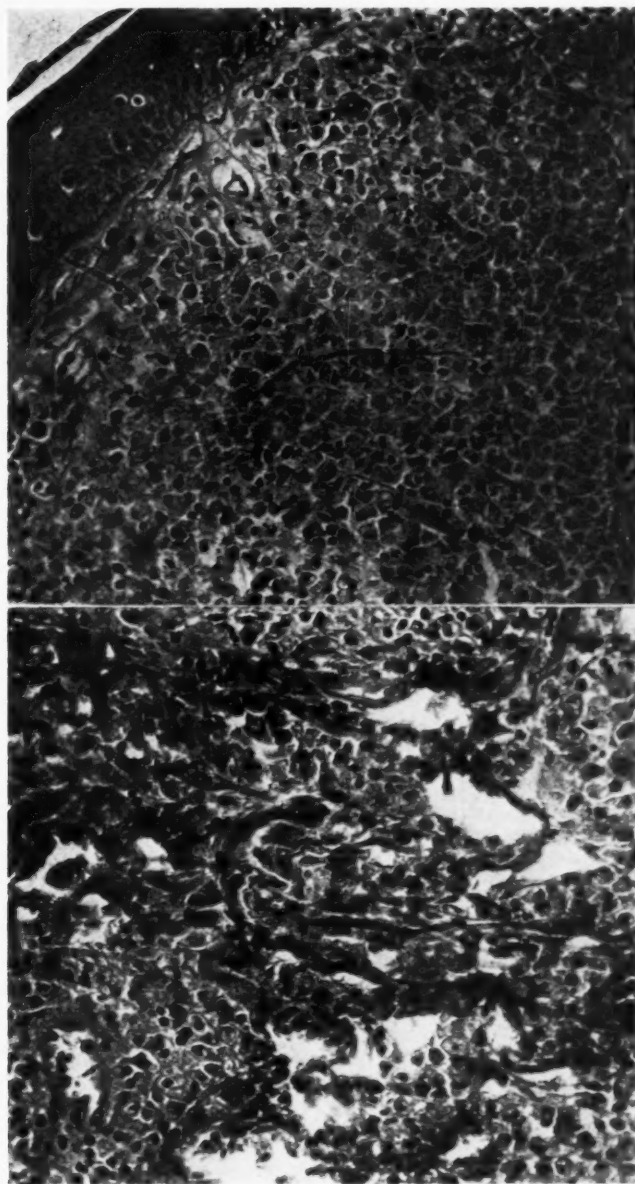


FIG. 2

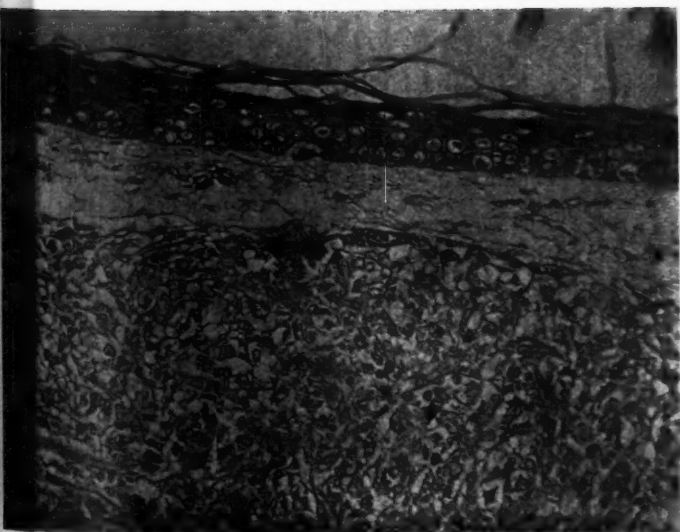


FIG. 3

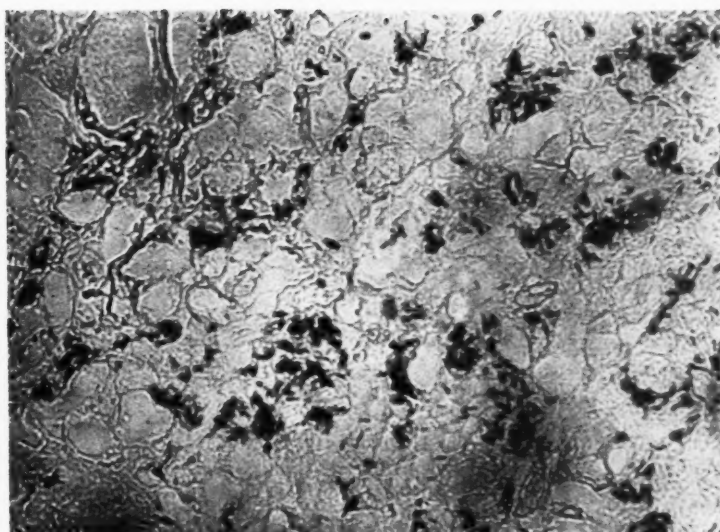


FIG. 4





## ANCYLOSTOMA BRAZILIENSE

BY

CLAYTON LANE

(Received for publication 2 September, 1922)

In a recent paper, Dr. Gordon (1922) reports finding *Ancylostoma braziliense* in man in four out of sixty-four autopsies performed in Manáos, Amazonas, Brazil, and concludes thus:—The comparison of these worms and other two-toothed ancylostomes from dogs and cats in North Brazil and India, and also from cats in South Africa and dogs in West Africa, failed to show the difference claimed to exist by de Faria between *A. ceylanicum* and *A. braziliense*.'

It is very desirable that a decision on the matter of identity of these worms should be generally accepted, and the first step necessary seems to be a historical survey rather fuller than that which Gordon supplies.

Gomes de Faria (1910) described *Ancylostoma braziliense* from *Felis domesticus* and *Canis familiaris* in Brazil. Looss (1911) described *Ancylostoma ceylanicum* from the civet cat, *Viverricula malacensis*, in Colombo, Ceylon. Leiper (1913), without examination of *A. braziliense*, suggested, from the appearance of the dorsal ray as figured by de Faria, that the two forms were identical, this ray having, he stated, a pair of digitations only on each of its two branches (Leiper (1915)), a statement which, however, requires alteration (Clayton Lane (1916)). Clayton Lane (1913) first recorded *A. ceylanicum* as a parasite of man, a fact since amply confirmed from various parts of the world, thereby giving to the question of nomenclature a medical interest. De Faria (1914) published a short paper in which he quotes a letter from Looss, who therein states emphatically that *A. braziliense* has only a single tooth on each ventral tooth plate; that its bursal rays, especially the externo-dorsal, are remarkable for their length and delicacy; and that the relative thickness of the bursal rays is a definite differential [specific] character. De Faria (1916), after examining abundant

Brazilian material and comparing it with specimens of *A. ceylanicum* sent by Clayton Lane from Bengal, verifies the existence of the inner pair of teeth, which he describes as much smaller than are those of the Indian forms, but holds, nevertheless, that this comparative examination disposes completely of Leiper's suggestion mentioned above. One of Looss's specific criteria being thus swept away, the specific differences held to obtain between the two forms rested upon the relative slenderness of the bursal rays. In this relation, Gordon published measurements of the externo-dorsal ray of Brazilian forms and of forms supplied to him from Bengal by Clayton Lane. These measurements provided him with no constant differences, nor could he detect other constant distinctions between worms from these two areas or from Africa.

The present intervention is prompted by two motives. The first is that the writer is credited by Gordon with supporting de Faria in his basis of specific differentiation. This is not exactly the case. What he actually did (Clayton Lane (1916)) was to comment upon the complete absence in existing descriptions of measurements of the internal organs; to express disbelief in Looss's statement that only a single pair of teeth existed, it being inconceivable that de Faria should describe and draw a non-existent tooth; to accept Looss's and de Faria's statements that the bursal rays of the Brazilian form were strikingly fine; and to point out that, accepting this as a fact, there emerged the almost certain conclusion that two species were being dealt with. There was this significant addition, 'It will probably be generally felt that there must be a thorough and independent examination by another experienced helminthologist before the question can be considered as settled.' This examination has been made by Gordon, but even his published report leaves certain matters doubtful. The receipt of some material furnished by his courtesy, together with the importance of settling definitely, if possible, the specific name of a parasite of man (the second of the motives to which reference was made above) prompts the present note.

An examination of the appended Table of Measurements mainly dealing with the internal organs of these forms, published apparently for the first time so far as the Brazilian ancylostomes are concerned, affords no justification for the duality of species. On



FIG. 1. *Ancylostoma braziliense*

Brazilian forms:

A, B, and C, the bursae of males.

D, E, and F, the tooth-plates of males.

Indian forms:

G, the bursa of the male.

H, I, and K, the tooth-plates; H and K of males, I of a female.

Scales:

L, scale for D, E, F, H, I, and K representing 0.1 mm.

M, scale for A, B, C, and G, representing 0.1 mm.

the other hand, it gives no proof of unity of species, as is clear when one considers, for example, the relative measurements of the various members of the genus *Trichostrongylus*. This question must under the circumstances be determined by shape, as, indeed, should always be the case.

Taking first the male bursa: Figs. A, B, and C are from Gordon's Brazilian material; fig. G from the dog in Bengal. Fig. B is typical of the stout-rayed condition generally ascribed to *A. ceylanicum*. In fig. A the rays are much finer. Fig. C shows a condition on the whole intermediate between the other two, although the externo-dorsal ray is short, ending far from the edge of the bursa, while the ventral rays and the internally-terminating lateral ones are pointed. This evidence demonstrates considerable individual variation upon those very points which are held constant within the species. This circumstance led naturally to a re-examination of Indian material. Almost at once the form represented in fig. G was found. Its relatively fine lateral rays are not those associated with the accepted descriptions of *A. ceylanicum*, and yet they are from Indian material.

Turning to the ventral oral plate, figs. D, E and F are from Gordon's Brazilian material. The first shows a direct dorsal view from a male worm, with the inner, deeper teeth fairly marked. Fig. E is that of another male viewed dorso-laterally; the obliquity brings into evidence and increases the apparent size of one deep tooth and obscures and minimises the other. In fig. F, clearing in creosote of this worm, which had lain long in lacto-phenol, was unsatisfactory and the deep teeth were invisible in a direct dorsal view. With lateral tilting their points could just be distinguished. Figs. B and F are from one and the same worm, apparently stout bursal rays being associated with apparent absence of the inner teeth. Figs. H, I and K are from Indian material, fig. I being from a female worm. Fig. K corresponds to fig. F. In it the inner tooth on one side is completely, and on the other almost completely, hidden by the large superficial outer tooth, and, had the specimen been imperfectly cleared, these would have been invisible.

The evidence which has just been given shows that the bursal rays of these two-toothed forms, from whatever part of the world



they come, present great individual differences in length and width, the former partly real, partly apparent, and due to the fore-shortening caused by the incurving of the bursal edge; and that marked variations occur in the apparent size of the inner teeth, variations which can indeed, to some extent at least, be produced at will by the rolling of the worm. Both features are largely independent of the country of origin. Indeed, one must conclude that individual prepossession will play a preponderating part in determining whether any particular two-toothed ancylostome of this type is to be classified as *A. braziliense* or *A. ceylanicum*. In other words, there is no evidence offered that acceptable specific differences exist between individuals from the Old and New Worlds. In the absence of such evidence, *Ancylostoma* (*Ceylancylostoma*) *ceylanicum* (Looss (1911)) lapses as a synonym of *Ancylostoma* (*Ceylancylostoma*) *braziliense* (Gomes de Faria, (1910)).

TABLE

Measurements in millimetres of Males of *Ancylostoma braziliense* from Brazil, and *A. ceylanicum* from Bengal.

	Brazilian form	Indian form
Oral cavity, length ... ..	0.14	0.143
Oral cavity, transverse diameter ... ..	0.08	0.09
Oral cavity, dorso-ventral diameter ... ..	0.07	0.087
Nerve collar from head end ... ..	0.7	0.57
Cervical papillae „ ... ..	0.55	0.57
Excretory pore „ ... ..	0.55	0.57
Width of cuticular striation ... ..	0.007	0.0075
Oesophagus, length ... ..	0.6	0.7
Oesophagus, breadth ... ..	0.1	0.15
Length of spicules ... ..	0.8 to 0.9	0.8
Length of accessory piece ... ..	0.065	0.075
Length of Cement gland ... ..	2.0	3.0

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# INTRA-UTERINE INFECTION WITH *ANCYLOSTOMA CANINUM* IN DOGS

BY

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AND

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*(Received for publication 3 September, 1922)*

Intra-uterine infection with hookworms has been noted by Howard (1917), who found ova in the stool of a child fourteen days old.

Owing to lack of human material, we examined a number of young animals in order to find whether intra-uterine infection with hookworms is a common occurrence.

Thirteen young dogs (from two to fifteen days old), representing eight different litters, were examined for ancylostomes. The results were as follows:—

Litter	Age in days of dogs	Number examined	<i>A. caninum</i>		Remarks
			Worms	Ova	
1	2	2	negative	negative	One infection was intense. Ancylostomes up to 7 mm. long with well developed buccal capsules. No ova in the uteri of the worms.
2	5	2	negative	negative	
3	5	2	positive (2)	negative	
4	7	2	negative	negative	
5	13	1	positive	positive	
6	14	2	positive (2)	positive (2)	
7	14	1	negative	negative	
8	15	1	positive	positive	

It thus appears that in Freetown, where intense infections with *A. caninum* are the rule in dogs, intra-uterine infection is common.

It is noteworthy that, although infection with *A. ceylanicum* is common in adult dogs, we have not found evidence of intra-uterine infection with this parasite.

Infection of the foetus is possible in two ways:—

(1) By larvae passing through the maternal blood stream to the placenta, and through the placenta to the foetus.

(2) By larvae finding their way into the peritoneal cavity of the mother and passing through the uterine muscle to the placenta.

Yoshida (1920) has shown the possibility of this by observing ancylostome larvae in the peritoneal cavity of experimentally infected guinea-pigs; and we have found ancylostome larvae in the peritoneal cavity of a guinea-pig which had been placed for ten hours in a vessel containing a mixed culture of *A. caninum* and *A. ceylanicum*.

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# CESTODES FROM INDIAN BIRDS WITH A NOTE ON *LIGULA INTESTINALIS*

BY

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A few species of cestodes dealt with below were presented to the author by Lt.-Col. Clayton Lane, I.M.S. The rest of the collection (except *Ligula*) were obtained from animals which died in the Zoological Gardens, Calcutta, on which post mortems were made in the Indian Museum.

The following species are recorded in this paper:—

PARASITE	HOST
<i>Tetrabothrius erostris</i>	<i>Sterna bergii</i>
<i>Davainea tetragona</i>	<i>Pavo muticus</i>
" "	<i>Pavo cristatus</i>
" "	<i>Francolinus vulgaris</i>
<i>Davainea</i> (? <i>tetragona</i> )	<i>Pavo nigropennis</i>
<i>Davainea microscolecina</i>	<i>Eclectus vioratus</i>
" "	<i>Eos ricinata</i>
<i>Davainea polychalix</i>	<i>Lorius garrulus</i>
<i>Davainea cruciata</i>	<i>Pica rustica</i>
<i>Davainea</i> sp.	Crow pheasant
<i>Davainea urogalli</i>	Tragopan pheasant
<i>Davainea tragopani</i> , n.sp.	Tragopan pheasant
<i>Davainea centropi</i> , n.sp.	<i>Centropus rufipennis</i>
<i>Cotugnia fastigata</i>	<i>Ptistis coccineopterus</i>
<i>Dilepis cypselina</i>	<i>Dendrocitta leucogaster</i>
<i>Dilepis campylancristrota</i>	<i>Herodias garzetta</i>
" "	<i>Ardeola grayi</i>
<i>Choanotaenia decacantha</i>	<i>Gallinago</i> sp.
<i>Choanotaenia</i> (? <i>octocantha</i> )	Snipe
<i>Choanotaenia microsoma</i> , n.sp.	<i>Ploceus atrigula</i>
" "	<i>Melophus melanicterus</i>
<i>Cyclorchida omalancristrota</i>	<i>Platalea</i> sp.
<i>Rhabdometra tomica</i>	<i>Francolinus pictus</i>
<i>Hymenolepis medici</i>	<i>Pelicanus philippensis</i>
" <i>fuscus</i>	<i>Larus brunneicephalus</i>
" "	<i>Hydropogon caspia</i>
" <i>lanceolata</i>	<i>Chenopsis atrata</i>



PARASITE	HOST
<i>Hymenolepis lanceolata</i>	<i>Cygnus atratus</i>
" "	Black swan
" <i>naja</i>	<i>Copschychus saularis</i>
" "	<i>Sitta chinensis</i>
" <i>zosteropsis</i>	<i>Criniger flaveolus</i>
" "	<i>Melophus melanicterus</i>
" "	<i>Closa (?) chinensis</i>
" "	<i>Ploceus atrigula</i>
" "	<i>Dendrocitta</i> sp.
<i>Hymenolepis farciminalis</i>	<i>Pica rustica</i>
" <i>stylosa</i>	<i>Brachypternus aurantius</i>
" "	<i>Trochalopterum meridionale</i>
" "	<i>Pica rustica</i>
" <i>asymetrica</i>	<i>Urocissa occipitalis</i>
" (? <i>microcephala</i> )	<i>Ciconia alba</i>
" (? <i>simplex</i> )	<i>Tadorna cornuta</i>
" sp.	<i>Emberiza luteola</i>
" "	<i>Garrulax belangeri</i>
" "	<i>Oriolus melanocephalus</i>
" "	<i>Liothrix lutia</i>
" "	<i>Dendrocitta rufa</i>
" "	<i>Tadorna cornuta</i>
" <i>annandalei</i> n.sp.	<i>Limosa belgicæ</i>
<i>Echinocotyle uralensis</i>	Snipe
" "	<i>Gallinago</i> sp.
<i>Hymenolepis capillaroides</i>	Snipe
<i>Diploposthe laevis</i>	<i>Netta rufina</i>
" "	<i>Nyroca ferina</i>
" sp. (? <i>laevis</i> )	<i>Streptilas interpres</i>
<i>Dioicocestus novae guineae</i>	<i>Podiceps albipennis</i>
<i>Cestode</i> sp.	<i>Sterna fluviatilis</i>
<i>Ligula intestinalis</i>	<i>Danio acquipinnatus</i>

Family TETRABOTHRIIDAE, Ransom, 1909

*Tetrabothrius erostris* (Loennberg, 1889), Führmann, 1899

Three specimens without heads from intestine of *Sterna bergii*. Lake Tamblegam, Ceylon, 6.9.12. Numbered Z.E.V.  $\frac{6047}{7}$  in the collection of the Indian Museum.

Family DAVAINIIDAE, Führmann, 1907

Sub-family DAVAININAE, Braun, 1900

*Davainea tetragona* (Molin, 1858), R. Blanchard, 1891

1. About fifty large specimens from intestine of *Pavo muticus*. Zoological Gardens, Calcutta. Collected by the author, 10.12.14.

2. About forty specimens, same host and locality. Collected by the author, 3.1.17.

3. About ninety specimens, same host and locality. Collected by the author, 4.4.18.

4. About twenty specimens, same host and locality. Collected by the author, 12.7.18.

5. Several large and complete specimens from *Pavo cristatus* (common pea-fowl). Zoological Gardens, Calcutta. Collected by the author, 17.4.18.

6. Two specimens without heads from intestine of black shouldered pea-fowl. Zoological Gardens, Calcutta. No date.

7. Several specimens from intestine of *Francolinus vulgaris* (black Francolin). Zoological Gardens, Calcutta. Collected by the author, 30.12.13.

Twelve entire specimens were mounted, and a number of detached heads. In many heads all the hooks had been lost. In others only the hooks on the suckers were missing; in still others some of the rostellar and sucker hooks were missing. Only in two or three heads were the hooks complete. In no case were the pores irregular, being invariably unilateral. Most of the strobilae were old and full of ripe eggs, but quite a number were ripe but not gravid. These measured from 5 mm. to 3 cms. in length.

*Davainea* (? *tetragona*)

A few fragments without head from intestine of *Pavo nigropennis* (black shouldered peacock). Collected by Lt.-Col. Clayton Lane, I.M.S., Berhampur, Bengal, 15.5.12.

*Davainea microcolecina*, Führmann, 1908

1. Five specimens from intestine of *Eclectus vioratus* (parrot). Zoological Gardens, Calcutta. Collected by the author, 22.1.14. Previously recorded from *Eclectus rosatus*.

2. Two specimens from intestine of *Eos ricinata*. Zoological Gardens, Calcutta. Collected by the author, 6.7.15.

Some of these specimens shewed a number of ripe segments strongly impregnated with lime. As a result they would not clear in clove oil, but after decalcifying in acid alcohol for several days they cleared readily. This phenomenon was often noted whilst working out the collection of Indian *Cestoda*.

*Davainea polychalix*, Kotlán, 1920

1. Four specimens from intestine of *Lorius garrulus*. Zoological Gardens, Calcutta. Collected by the author, 15.3.17.
2. Two specimens, same host and locality. Collected by the author, 13.3.17.

*Davainea cruciata* (Rud. 1819), Führmann, 1908

One specimen from intestine of *Pica rustica* (magpie). Zoological Gardens, Calcutta. No date.

*Davainea* sp.

A few fragments without heads from intestine of a crow pheasant. Zoological Gardens, Calcutta. Collected by the author, 22.4.15.

*Davainea urogalli* (Modeer, 1790), R. Blanchard, 1891

One specimen and several fragments from intestine of a Tragopan pheasant. Zoological Gardens, Calcutta. Collected by the author, 27.2.15.

In these specimens the head was about  $380\mu$  broad; its length could not be accurately determined because it passed into the neck, but it appeared to be at least  $500\mu$ . The suckers have a diameter of about  $150\mu$  and are armed with about 17 rows of hooks. The rostellum has a diameter of about  $50\mu$ , and is armed with a double row, each hook measuring 7 or  $8\mu$ . About 50 were counted, but a number of hooks had clearly been lost. The total number is probably less than 100.

The muscular system is feebly developed and consists of a few scattered longitudinal fibres, internal to which there occur a few transverse strands.

The ventral excretory vessels on each side are very large, having a diameter of about  $120\mu$ . They communicate with each other transversely by an equally large tube, in the posterior part of each segment. The dorsal vessel on each side is minute and has a diameter of  $10\mu$  only.

The parenchyma throughout the worm is greatly developed, and it is very spongy owing to the occurrence of numerous small excretory cavities.

The pores are unilateral and are situated in the anterior half of the segment. The testes number about 36-40; nine or ten are situated on the pore side and the rest posterior to the ovary, and aporal. Each testis has a diameter of about  $55\mu$  when mature. In full development they

extend from the dorsal to the ventral surfaces and from the anterior to the posterior margins. They lie strictly within the water vessels.

The cirrus pouch lies across the antero-lateral angle and extends to the water vessel.

The vagina is posterior to the cirrus pouch. Both the genital canals run between the dorsal and ventral excretory vessels.

Meggitt (1921) states that a number of eggs occur in each capsule, whilst Shipley (1909) states that the eggs lie singly in the parenchyma. In the Indian species the eggs at first occur in numbers in each capsule, but when fully developed each capsule contains only one onchosphere.

Führmann states that the eggs lie within the two ventral water vessels. In our specimens, sections shewed that a single discontinuous layer of eggs was closely adherent to the lateral wall of each vessel, but they did not extend beyond that limit.

#### *DAVAINEA TRAGOPANI*, n.sp.

Two specimens from intestine of a Tragopan pheasant. Zoological Gardens, Calcutta. Collected by the author, 27.2.15.

#### EXTERNAL ANATOMY

Only one of the specimens possessed a head. This worm measured 8.5 mm. in length, and its greatest breadth was  $600\mu$ . It was composed of 27 or 28 segments; the last segment measured  $825\mu$  in length and  $600\mu$  in breadth. The second specimen (without head) measured 7 mm. in length and its greatest breadth was  $600\mu$ . It contained 27 or 28 segments.

*Head.* This was  $180\mu$  broad and about  $125\mu$  long. Without destroying the head it was impossible to obtain accurate details relating to the hooks, but 23 hooks were counted in what appeared to be half the circumference of the rostellum. It seems, therefore, that the total number of hooks present was about 46. They did not appear to be in a double row. Their exact shape could not be made out, but they appeared to be typical. They measured  $10\mu$  in length. The suckers are armed, but all the hooks had been lost except in a portion of one sucker, where there appeared to be from 4 to 6 rows.

The neck measured about  $300\mu$  in length and was present in both specimens.

## INTERNAL ANATOMY

Owing to lack of material the nervous, muscular, and excretory systems were not investigated.

*Genitalia. Testes.* There are 6 or 7 testes and they first appear in about Segment IV. When fully mature they measure about  $70\mu$ . Usually there are four situated aporally, one or two posterior to the ovary, and a single testis on the pore side, posterior to the internal extremity of the cirrus.

*Vas deferens.* The cirrus pouch when fully developed extends half-way across the segment; in 2 or 3 cases it extends a little more than half-way across. It has very thick (? muscular) walls. In Segment XVII it measures  $250\mu$  long and  $110\mu$  broad. The cirrus is peculiar in being

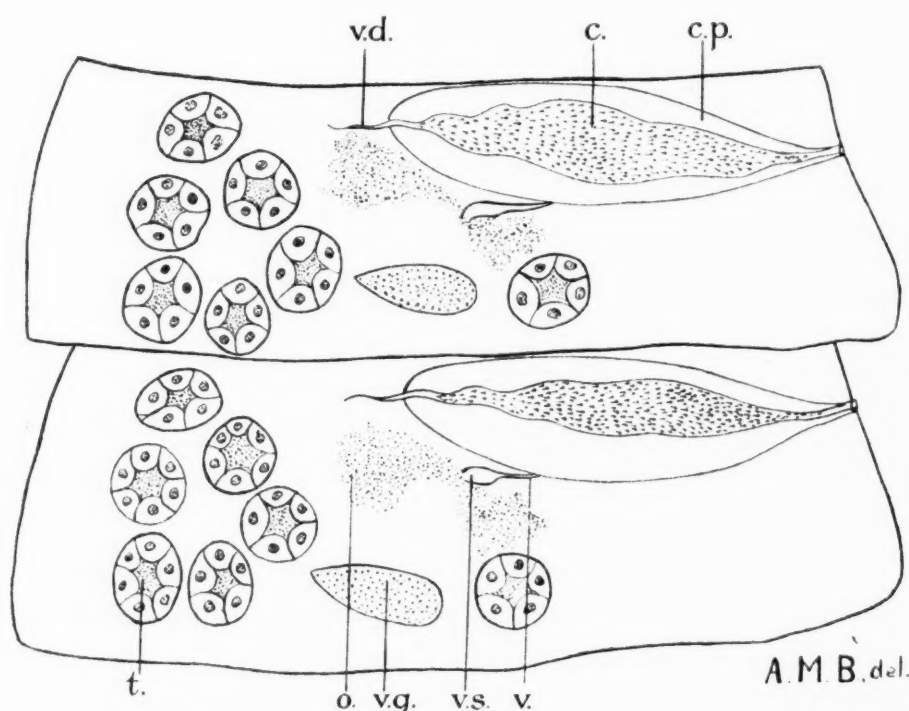


FIG. 1. *Davainea tragopani*, n.sp. Ripe segments, mounted whole, showing genitalia. c.—cirrus; c.p.—cirrus pouch; o.—ovary; t.—testes; v.—vagina; v.d.—vas deferens; v.g.—vitelline gland; v.s.—receptaculum seminis.  $\times 210$ .

a greatly dilated organ densely covered with minute spines, and almost filling the cirrus pouch. The cirrus pouch persists to the last segment. The vas deferens is short and very slightly coiled. No seminal vesicle was observed (fig. 1).

The genital pores are unilateral and are situated a little anterior to the middle point of the lateral margin of each segment.



*Ovary.* The ovary, which first appears in about Segment VIII, is definitely bilobed, each lobe being globular, and composed of a number of rounded acini. In full development each lobe measures about  $70\mu$  in diameter.

*Receptaculum and vagina.* From the pore the vagina pursues a direct course to a point between the two lobes of the ovary where it dilates into a receptaculum seminis.

*Vitelline gland.* This lies posterior to the ovary and is a conspicuous organ. In full development its transverse and anterior diameters measure about  $60\mu$  (fig. 1).

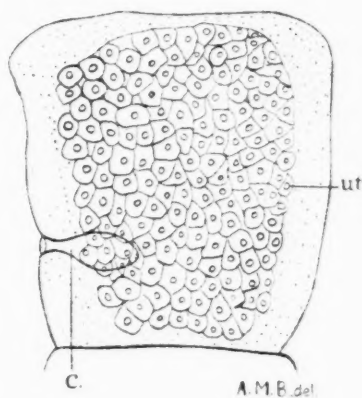


FIG. 2. *Davainea tragopani*, n.sp. Gravid segment, mounted whole, showing uterus. c.—cirrus pouch; ut.—uterus.  $\times 50$ .

*Uterus.* This first appears as a small cavity immediately anterior to, and between, the two lobes of the ovary. It enlarges and eventually single eggs become isolated in the parenchyma. In the last few segments no trace of the excretory vessels could be seen in either specimen; it is, therefore, impossible to say definitely whether the eggs extend beyond them or not. But as there was a definite area between the edge of the segment and the eggs, it would appear that the latter lie internal to the excretory vessel (fig. 2).

*Eggs.* These have a diameter of about  $54\mu$  and the onchosphere of about  $25\mu$ .

#### DIAGNOSIS

The species is related to the *proglottina* type. The following table gives details of the various species described which resemble *D. proglottina* in being of small size, and at the same time serves to shew the points in which *D. tragopani* differs from related species. I have, unfortunately,

been unable to procure Kowalewsky's paper. Führmann recently (1919) discussed the relationship of the first four species indicated in the table, and it would appear almost certain that *D. varians*, *D. dubius* and *D. proglottina* var. *dublanensis* are synonyms of *D. proglottina*.

The principal points in which *D. tragopani*, n.sp., differs from them all are :—(1) size ; (2) number of segments ; and (3) the unilateral pores.

The type specimen has been returned to the Indian Museum, Calcutta.

TABLE I.

	Length	Breadth	No. of Segments	No. of Hooks	Size of Hooks	Suckers	Pores	Testes	Eggs
<i>D. proglottina</i> ...	mm. 1·5	mm. 0·5	2-5	80-95	6 $\mu$	1 row armed	regularly alternate	22 on one side	35 $\mu$
<i>D. varians</i> ...	1·8	?	4-6	44-50	?	4-5 rows armed	regularly alternate	more than 10	?
<i>D. dubius</i> ...	3·3	0·63	7-9	2 rows 50-60	7·1-8·4 $\mu$	4-6 rows armed	alternate	12-15	330 onch. 230
<i>D. dublanensis</i> ...	4·0	?	6	...	?	armed ?	irregularly alternate	?	?
<i>D. tetraoensis</i> ...	2·3	0·35	9-10	2 rows 120-130	9 $\mu$	armed with several rings	alternate	about 30	onch. 270
<i>D. minuta</i> ...	1·0	0·4	8	?	9 $\mu$	unarmed	alternate	10-12	?
<i>D. paucisegmentata</i> ...	5·0	0·7	5	?	?	unarmed	unilateral	40	160
<i>D. bimantopodis</i> ...	1·0	?	7-8	2 rows 50	7 $\mu$	armed, no neck	irregularly alternate	4	230
<i>D. tragopani</i> n.sp. ...	8·0	0·6	27	2 rows 46	10 $\mu$	4 rows, armed	unilateral	6	540 onch. 250

#### DAVAINEA CENTROPI, n.sp.

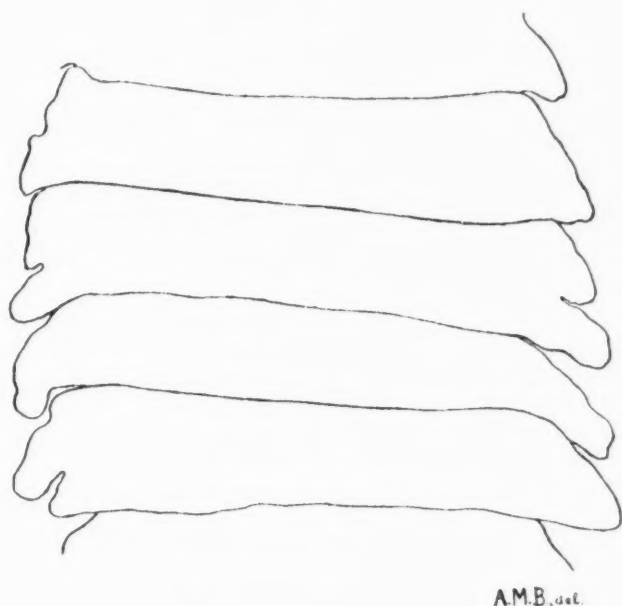
Three specimens and two fragments from intestine of *Centropus rufipennis* (the common Caccal), Lake Tamblegam, Ceylon, October 1911. Numbered Z.E.V.  $\frac{6103}{7}$  in the collection of the Indian Museum.

## EXTERNAL ANATOMY

The specimens measured from 2.5 cms. to 3.5 cms. in length and had a maximum breadth of about 1.5 mm.

*Head.* The head is prominent and presents a truncated appearance; it measured about  $300\mu$  broad. Its length could not be determined owing to the fact that it merges into a very short neck. The suckers have a diameter of about  $300\mu$ ; each sucker bears on its margin about 15 rows of hooks each measuring about  $8\mu$ . The rostellum is relatively small and is armed with about 300 hooks measuring from  $9\mu$  to  $11\mu$  in length and arranged in a double row.

*Segments.* The segments are very much broader than long, all except a few at the posterior extremity being quite shallow. Their lateral posterior margins are produced as shown in fig. 3. The genital pores are irregularly alternate being situated, and directed, anteriorly.



A.M.B., del.

FIG. 3. *Davainea centropi*, n.sp. Outline of four segments.  $\times 35$ .

## INTERNAL ANATOMY

*Muscular system.* This system is poorly developed; the longitudinal fibres are relatively scanty and consist of small bundles somewhat widely separated; the bundles decrease in size externally. The transverse fibres lie internal to the longitudinal muscles and are also very scanty. No oblique or dorso-ventral fibres were seen (fig. 4).

*Nervous system.* A small single nerve strand was to be seen lateral to the ventral water vessel on each side. On the pore side the nerve was ventral to the cirrus pouch and vagina.

*Excretory system.* This consists of a single ventral vessel on each side; on the pore side it lies ventral to the cirrus pouch (fig. 4).

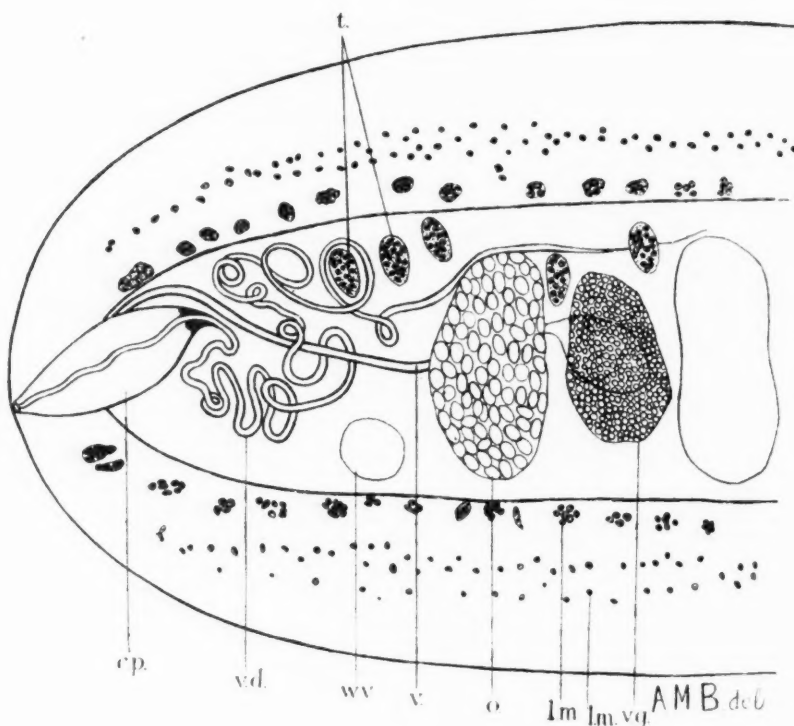


FIG. 4. *Davainca centropi*, n.sp. Transverse section showing cirrus pouch, vas deferens, vagina, ovary and muscular system. *c.p.*—cirrus pouch; *l.m.*—longitudinal muscle; *o.*—ovary; *t.*—testes; *v.*—vagina; *v.d.*—vas deferens; *v.g.*—vitelline gland; *w.v.*—water vessel.  $\times 130$ .

*Genitalia. Testes.* The testes are about forty in number; they lie dorsal and anterior on each side of the ovary and extend beyond the ventral excretory vessel. They are somewhat oval in shape and, when fully developed, measure about  $85\mu$  by  $55\mu$ .

*Vas deferens.* The vas deferens is remarkable in being very long. It extends half-way across the segment and is thrown into a large number of loops which occupy almost the entire field between the internal extremity of the cirrus pouch and the poral wing of the ovary. No seminal vesicle was observed. The cirrus pouch varies in length, extending from about half to three-quarters the distance between the lateral margin and the ventral excretory vessel (fig. 4).

*Ovary.* The ovary is a relatively large bi-lobed organ lying ventral

and posterior; in full development it extends almost to the dorsal transverse muscle fibres (figs. 4 and 5).

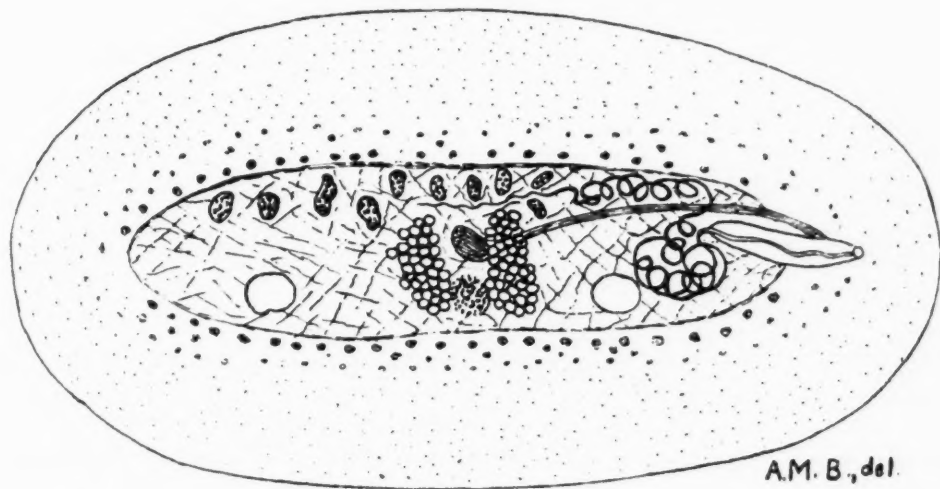


FIG. 5. *Davainea centropi*, n.sp. Transverse section showing male and female genitalia.  $\times 70$ .

*Receptaculum and vagina.* From the pore, the vagina runs dorsal to the cirrus pouch; at the internal extremity of the latter organ, the vagina curves gradually and runs directly to the ovary. It is muscular throughout its length. Its internal extremity is dilated into a muscular receptaculum seminis, which, in full development, measures about  $150\mu$  in length and  $50\mu$  in breadth (fig. 4). The oviduct, vitelline duct, and fertilisation canal are noticeable on account of their length.

*Vitelline gland.* This lies ventral to and between the two lobes of the ovary; it is large and easily seen (figs. 4 and 5).

*Uterus.* In full development, the uterus extends beyond the ventral excretory vessels and consists of a large number of parenchymatous capsules, each containing a single onchosphere.

*Eggs.* These have a diameter of about  $55\mu$ ; the onchosphere measures about  $36\mu$ .

#### DIAGNOSIS

Up to the present only about fourteen species of *Davainea* have been recorded which have armed suckers, and irregularly alternating genital pores. The species just described differs very definitely from them all. I therefore consider the species new and have named it *D. centropi*.



*Cotugnia fastigata*, Meggitt, 1920

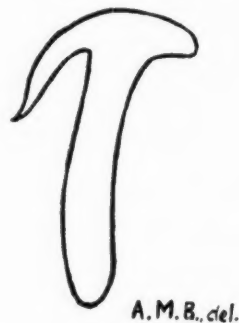
Three specimens from intestine of *Ptistes coccineopterus*, Gould, 1865, Zoological Gardens, Calcutta. Collected by the author, 13.11.15.

The specimens had the following measurements :—

TABLE II.

	1	2	3
Length ... ..	75·0 mm.	70·0 mm.	60·0 mm.
Greatest breadth ... ..	3·5 mm.	3·8 mm.	3·3 mm.
Number of segments ... ..	212	210	205

As Meggitt was unable to isolate and figure a complete rostellar hook, a drawing of a hook from the Indian example is given below (fig. 6).

FIG. 6. *Cotugnia fastigata*, Meggitt. Diagram of a hook.

In our specimens the vagina was almost invariably situated some distance posterior to the cirrus pouch, and as a result it was impossible to determine whether the vagina was dorsal or ventral to the pouch. In 6 or 7 segments examined, however, the vagina was definitely dorsal to the pouch on one side and ventral on the other—a character peculiar to the genus *Moniezia*.

Family *HYMENOLEPIDIDAE*, Railliet and Henry, 1909

Sub-family *DIPYLIDIINAE*, Stiles, 1896

*Dilepis cypselina*, Neslobinsky, 1911

One fragment with a head, of what is almost certainly this species, was obtained from the intestine of *Dendrocitta leucogaster* (tree-pie) ; Zoological Gardens, Calcutta. Collected by the author, 7.12.15.

The head was armed with a double crown of about 90 hooks, measuring about  $24\mu$ . The genital pores were unilateral. The cirrus pouch was situated anteriorly, and extended almost to the water vessel.

*Dilepis campylancristrota* (Wedl, 1855), Führmann, 1908

1. Four specimens from intestine of *Herodias garzetta* (paddy bird), Berhampore, Bengal. Collected by Lt.-Col. Clayton Lane, I.M.S., June, 1912. Numbered Z.E.V.  $\frac{6019}{7}$  in the collection of the Indian Museum.

2. Numerous specimens from *Ardeola grayi* (pond heron), Zoological Gardens, Calcutta. Collected by the author, 14.12.13, and numbered Z.E.V.  $\frac{6161}{7}$  in the collection of the Indian Museum.

*Choanotaenia decacantha*, Führmann, 1913

Four specimens from intestine of a snipe (*Gallinago* sp.), Berhampur, Bengal. Collected by Lt.-Col. Clayton Lane, I.M.S., 17.12.12.

The specimens agreed with Führmann's description except in the following minor details:—

(1.) The hooks measured  $23.4\mu$ ; in the type specimen they measured  $19.8\mu$  to  $21.6\mu$ .

(2.) The type specimen had from 40 to 50 segments; the Indian forms have from 40 to 98 segments.

*Choanotaenia* (? *octocantha*, Führmann)

1. One specimen, without head, from intestine of a snipe, Berhampur, Bengal. Collected by Lt.-Col. Clayton Lane, I.M.S., 12.3.12.

2. One specimen from same host and locality. Collected by Lt.-Col. Clayton Lane, I.M.S. (219 b), 17.12.12.

#### *CHOANOTAENIA MICROSOMA*, n.sp.

1. Six specimens from intestine of *Ploceus atrigula* (the eastern baya). Zoological Gardens, Calcutta. Collected by the author, 26.10.15.

2. About twelve specimens from intestine of *Melophus melanicterus* (the crested bunting). Zoological Gardens, Calcutta. Collected by the author, 25.6.15

## EXTERNAL ANATOMY

The worms measure from 4 mm. to 8 mm. in length and have a maximum breadth of about  $630\mu$ . They consist of from 25 to about 50 segments.

*Head.* The head is square and measures about  $220\mu$ ; the suckers have a diameter of about  $140\mu$ . The rostellum measures about  $180\mu$  in length and has a diameter of about  $50\mu$ . Its anterior extremity is expanded and has a breadth of about  $90\mu$  and a length of  $40\mu$ . It is armed with a single row of from 16 to 20 hooks which measure about  $35\mu$  (fig. 7).

There is no neck.

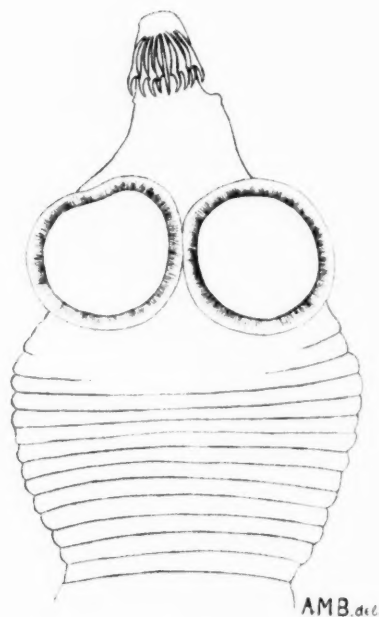


FIG. 7. *Choanotaenia microsoma*, n.sp. Head and anterior segments.  $\times 170$ .

## INTERNAL ANATOMY

*Muscular, excretory and nervous systems.* As the material was not sufficiently well preserved details of these systems are not obtainable.

*Genitalia. Testes.* There are from 16 to 20 testes situated posterior to the ovary. When fully mature they have a diameter of about  $36\mu$  (fig. 8).

*Vas deferens.* The genital pore is situated at the extreme anterior lateral angle of the segment and is very large and prominent. The cirrus pouch is short and narrow, extending to the water vessel to which it is dorsal. It lies anterior to the vagina. The cirrus is remarkable in

having its extreme tip armed with short spines set at right angles to its length. Immediately median to the tip, the cirrus is armed with a number of hooks of a different shape which measure  $30\mu$  in length, and which lie

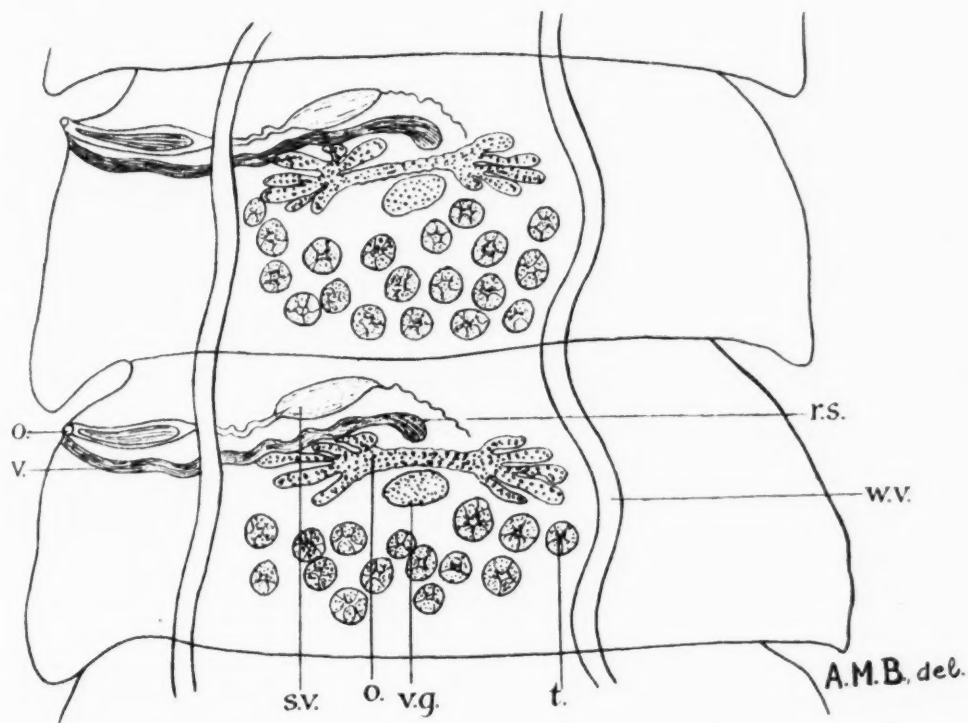


FIG. 8. *Cboanotaenia microsoma*, n.sp. Horizontal section showing male and female genitalia. o.—ovary; r.s.—receptaculum seminis; s.v.—seminal vesicle; t.—testes; v.g.—vitelline gland; w.v.—water vessel.  $\times 230$ .

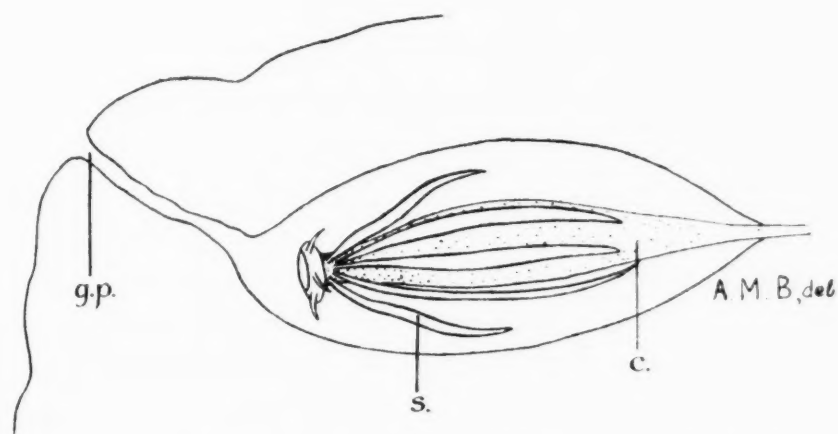


FIG. 9. *Cboanotaenia microsoma*, n.sp. Showing spines on cirrus. c.—cirrus; g.p.—genital pore; s.—spine.  $\times 750$ .

parallel to the cirrus (fig 9). The vas deferens dilates close to the median extremity of the cirrus pouch into a small seminal vesicle, and then continues in the median direction as a very fine tube (fig. 8).

*Ovary.* This organ lies quite anterior and is divided into two sets of acini, one on each side, widely separated from each other (fig. 8).

*Receptaculum and vagina.* The vagina is a wide muscular tube running posterior to the cirrus pouch and dorsal to the excretory vessel. Near the centre of the segment it dilates into a globular receptaculum, having a diameter of about  $36\mu$  (fig. 8).

*Vitelline gland.* This is a compact, deeply-staining organ lying posterior to a line joining the two wings of the ovary. It has a breadth of about  $110\mu$  (fig. 8).

*Shell gland.* This lies immediately anterior to the vitelline gland. It is somewhat globular and has a diameter of about  $30\mu$ .

*Uterus.* The uterus appears suddenly as a transverse sac situated in front of the ovary. In the next segment the ovary and testes have entirely and as suddenly disappeared, the whole segment being occupied by the uterus which extends beyond the water vessels. The eggs lie in capsules, one in each capsule.

#### DIAGNOSIS

The characters which distinguish this worm from other species of the genus *Choanotaenia* are: (1) its small size; (2) the small number of segments; (3) the number, size and shape of the hooks; (4) the peculiarly armed cirrus.

On account of its small size I have named it *Choanotaenia microsoma*.

*Cyclorchida omalancristota* (Wedl, 1856), Führmann, 1907

Several specimens from intestine of *Platalea* sp. (spoon bill). Zoological Gardens, Calcutta. Collected by the author, 21.11.13.

#### Sub-family *PARUTERININAE*, Ransom, 1909

*Rhabdometra tomica*, Cholodovsky, 1906

Two specimens from intestine of *Francolinus pictus* (painted partridge). Zoological Gardens, Calcutta. Collected by the author, 26.3.14.

The number of testes and the arrangement of the transverse and longitudinal muscle fibres left no doubt as to the identification of this species.



Sub-family *HYMENOLEPIDINAE*. Ransom, 1909

*Hymenolepis medici* (Stoss., 1890), Führmann, 1906

Several specimens from intestine of *Pelicanus philippensis*. Zoological Gardens, Calcutta, 18.9.19.

*Hymenolepis fusus* (Krabbe, 1869), Führmann, 1906.

1. A large number of specimens from *Larus brunneicephalus*. Zoological Gardens, Calcutta. Collected by the author, 22.1.17.

The hooks varied in size from  $12\mu$  to  $18\mu$ . It is important to note that of five worms examined, all of them shewed three or four segments with only two testes.

2. A large number of specimens from *Hydropogone caspia* (tern). Zoological Gardens, Calcutta. Collected by the author, 17.2.15.

In six of these specimens it was found that the number of testes was not constant, many segments possessing only two.

*Hymenolepis lanceolata* (Bloch, 1782), (Weinland, 1858), Braun, 1903

1. Six small specimens 1 cm. long, without heads, from the Black Australian Swan, *Chenopsis atrata*, Berhampur, Bengal, numbered Z.E.V.  $\frac{6050}{7}$  in the collection of the Indian Museum. Collected by Lt.-Col. Clayton Lane, I M S, 11.4.12.

2. About sixteen small specimens 2 to 3 cms. in length from same host. Zoological Gardens, Calcutta. Collected by the author, 24.4.18.

3. About twelve large specimens, 4 to 6 cms. in length and 1 cm. in breadth from same host. Zoological Gardens, Calcutta, 24.4.19.

4. About twenty large specimens, about 6 cms. in length and three small specimens, 2 cms. in length from *Cygnus atratus*. Zoological Gardens, Calcutta, 22.12.19.

5. Four large specimens, 4 to 6 cms. in length from the Black Swan. Zoological Gardens, Calcutta, 25.5.19. The variability of this species is discussed by Maplestone and Southwell in *Ann. Trop. Med. & Parasit.*, June, 1922.

*Hymenolepis naja* (Duj, 1845), Führmann, 1906

1. Three fragments from intestine of *Copschychus saularis* (Magpie robin). Zoological Gardens, Calcutta. Collected by the author, 5.8.15. All the fragments were stained and mounted.

2. Two specimens, one with a head, from *Sitta chinensis* (green

magpie). Zoological Gardens, Calcutta. Collected by the author, 27.4.15. Both specimens were stained and mounted.

*Hymenolepis zosteropsis*, Führmann, 1918

1. A large number of specimens from *Criniger flaveolus* (white cheeked Bulbul). Zoological Gardens, Calcutta. Collected by the author, 26.12.19. Our specimens measured from 2 mm. to 4 mm. in length ; the hooks were very typical of the species.

2. About ten specimens from intestine of *Melophus melanicterus*, Zoological Gardens, Calcutta. Collected by the author, 25.6.15.

3. Four specimens from intestine of *Closa* (?) *chinensis* (green magpie). Zoological Gardens, Calcutta. Collected by the author, 28.4.15.

4. Five specimens from intestine of *Ploceus atrigula* (the eastern baya). Zoological Gardens, Calcutta. Collected by the author, 12.10.15.

5. Three specimens from intestine of *Melophus melanicterus* (the crested bunting). Zoological Gardens, Calcutta. Collected by the author, 25.6.15.

6. Six specimens from intestine of *Dendrocitta* sp. (tree-pie). Zoological Gardens, Calcutta. Collected by the author, 15.5.13., and numbered Z.E.V.  $\frac{5953}{7}$  in the collection of the Indian Museum.

*Hymenolepis farciminalis* (Batsch, 1786) (R. Blanchard, 1891),  
Führmann, 1906

Several specimens from intestine of *Pica rustica* (magpie). Zoological Gardens, Calcutta. Collected by the author, 10.7.18.

A striking feature of our specimens of this species was the fact that a single strobila contained segments with no testes, and segments with one, two, three or four testes, although most segments contained three. Another feature was that the testes in some segments were in line, in other segments there were two testes aporal and one poral, and vice versa. In fact their disposition was quite irregular.

*Hymenolepis stylosa* (Rud., 1810), Volz., 1899

1. Several specimens from intestine of *Brachypternus aurantius* (golden backed wood-pecker). Zoological Gardens, Calcutta. Collected by the author, 31.12.13.

2. Four specimens (only one with a head) from intestine of *Trochalopteron meridionale* (laughing thrush). Zoological Gardens, Calcutta. Collected by the author, 9.8.15.

3. Two young strobilae (2 cms. long) from intestine of *Pica rustica*. Zoological Gardens, Calcutta. Collected by the author, 10.7.18. These were mounted.

*Hymenolepis capillaroides*, Führmann, 1906

1. Three specimens (one with a head) from intestine of a snipe. Berhampur, Bengal. Collected by Lt.-Col. Clayton Lane, I.M.S., 21.7.12.

2. Two specimens, one with a head, same host and locality. Collected by Lt.-Col. Clayton Lane, I.M.S., 12.3.12.

*Hymenolepis* (? *asymetrica*), Führmann, 1918

Three badly preserved specimens, apparently of this species (only one with a head) from intestine of *Urocissa occipitalis* (red-billed blue magpie). Zoological Gardens, Calcutta. Collected by the author, 22.10.19

Führmann obtained the species from *Chalcococcyx plagosus*, New Guinea. His specimens measured 10 cms. in length and 1 mm. in breadth. The head was armed with 10 hooks 19 $\mu$  long, and of a peculiar shape. Our specimen was armed with exactly similar hooks of the same size. The Indian specimens measured 1 cm. only in length and were quite immature. In the posterior segments the testes were developing and the rudiments of the ovary could be seen.

*Hymenolepis* (? *microcephala*) (Rud, 1819), Führmann, 1906

Numerous specimens from intestine of *Ciconia alba* (white stork). Zoological Gardens, Calcutta. Collected by the author, 6.6.19.

*Hymenolepis* (? *simplex*)

1. Two fragments and one head from intestine of *Tadorna cornuta* (sheldrake). Zoological Gardens, Calcutta. Collected by the author, 26.3.15.

2. Numerous specimens without heads from same host and locality, 18.3.14.

*Hymenolepis* spp.

1. A few fragments of a small worm apparently about 12 mm. in length, from the intestine of *Emberiza luteola*. Zoological Gardens, Calcutta, 11.11.15. The fragments were in a bad state of preservation. No head was present; there appeared to be three testes in the segments examined.

2. Other fragments also without heads from intestine of *Garrulax belangeri*. Zoological Gardens, Calcutta, 1.5.19.
3. Still others from intestine of *Oriolus melanocephalus*. Zoological Gardens, Calcutta, 12.10.15.
4. One specimen without head from intestine of *Liothrix lutia* (red-billed Liothrix). Zoological Gardens, Calcutta. Collected by the author, 29.5.16. The specimen measured 30 mm. in length and 2.5 mm in breadth. Two testes were situated on one side and one on the other.
5. Fragments from intestine of *Dendrocitta rufa*. Zoological Gardens, Calcutta, 13.6.15.
6. Several specimens without heads from intestine of *Tadorna cornuta* (common sheldrake). Zoological Gardens, Calcutta. Collected by the author, 18.3.14.
7. Two specimens without heads from same host and locality. Collected by the author, 23.6.15. In both specimens the testes were irregular, the conditions being similar to those described for *H. farciminalis*.

#### *HYMENOLEPIS ANNANDALEI*, n.sp.

Two specimens from the intestine of *Limosa belgicae* (black-tailed godwit). Barkuda, Chilka Lake, Orissa, India. Collected by Dr. N. Annandale, 28.4.28.

#### EXTERNAL ANATOMY

The specimens had the following dimensions :—

	Length				Greatest breadth
1.	60 mm.	...	...	...	1.5 mm.
2.	103 mm.	...	...	...	2 mm.

The anterior part of the worm is attenuated and whip-like ; all the segments are broader than long, the posterior and lateral margins being salient. The genital pores all unilateral, and situated slightly anterior to the middle of the lateral margin.

*Head.* The head measures about  $180\mu$  in length and is  $150\mu$  broad ; the suckers have a diameter of about  $80\mu$ . The rostellum is a conspicuous organ armed with a single row of 10 hooks which measure about  $32\mu$  in length (fig. 10). Both in size and shape they closely resemble those of *H. brasiliense*, Führ.

The neck measures about 2 mm. in length.

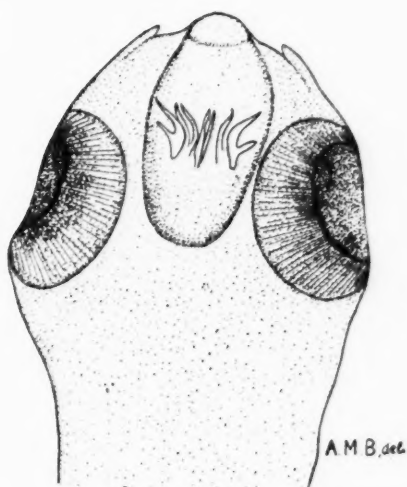


FIG. 10. *Hymenolepis annandalei*, n.sp. Showing head.  $\times 220$ .

#### INTERNAL ANATOMY

*Muscular system.* This is poorly developed. The longitudinal muscles consist of an inner and an outer series of bundles; the internal bundles are larger and fewer than the outer bundles, the latter being situated immediately beneath the cuticle. A few circular fibres occur between the outer and inner longitudinal bundles and also internal to the inner longitudinal fibres. No oblique fibres were seen (fig. 12).

*Nervous system.* Details of this system were not investigated. A small ill-defined nerve was observed in transverse sections, running external to the water vessel on each side.

*Water vascular system.* This consists of a single ventral vessel on each side, lying ventral to the cirrus pouch and vagina (fig. 12).

*Genitalia. Testes.* There were three testes; one is situated on the pore side and the other two are aporal, one being anterior to the other (figs. 11 and 12). When fully mature they have a diameter of about  $150\mu$  and occupy almost the whole of the segment dorso-ventrally.

*Vas deferens.* The cirrus pouch lies dorsal to the vagina; it is somewhat club-shaped, the broader extremity being median. It measures about  $180\mu$  in length and its greatest breadth is about  $40\mu$ . Its median half is occupied by an internal seminal vesicle. In the median direction it continues as a very short, wide, coiled tube and then dilates into a large external seminal vesicle which measures about  $160\mu$  in length and  $30\mu$  in breadth (fig. 12); the median extremity of the external seminal vesicle is close to the poral testis.



*Ovary.* The ovary is situated ventrally in the middle line, and posterior; it measures about  $300\mu$  broad and  $100\mu$  in the antero-posterior direction, whilst dorso-ventrally it practically fills the segment (fig. 11).

*Receptaculum and vagina.* The vagina is a very muscular organ measuring about  $450\mu$  in length and is club-shaped. At the pore its breadth is about  $10\mu$ ; it gradually widens and attains a maximum diameter of  $50\mu$  at a point opposite the middle of the external seminal vesicle. It then narrows gradually. The whole vagina functions as a receptaculum.

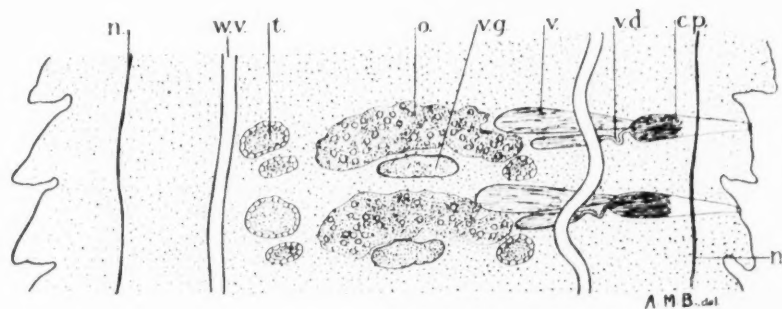


FIG. 11. *Hymenolepis annandalei*, n.sp. Horizontal section showing genitalia. c.p.—cirrus pouch; n.—nerve; o.—ovary; t.—testes; v.—vagina; v.d.—vas deferens; v.g.—vitelline gland; w.v.—water vessel.  $\times 60$ .

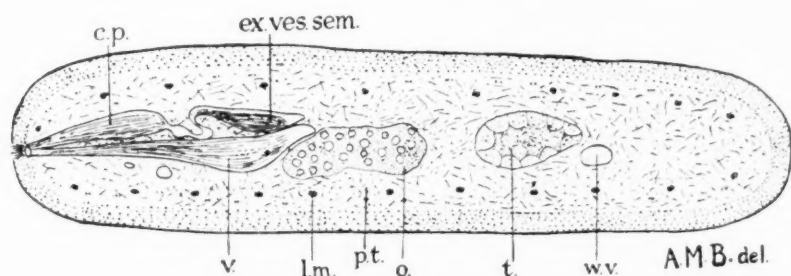


FIG. 12. *Hymenolepis annandalei*, n.sp. Transverse section showing cirrus pouch, vagina and the great development of parenchymatous tissue. c.p.—cirrus pouch; ex. ves. sem.—external vesicula seminalis; l.m.—longitudinal muscle; o.—ovary; p.t.—parenchymatous tissue; t.—testes; v.—vagina; w.v.—water vessel.  $\times 72$ .

*Vitelline gland.* This is a conspicuous bi-lobed organ situated posterior to the centre of the ovary. It is about  $100\mu$  broad (fig. 11).

*Uterus.* The uterus consists of a simple transverse sac extending well beyond the water vessel on each side, and almost to the edge of the segment. The eggs were not mature; the largest measured  $17\mu$  in diameter and the onchosphere measured  $11\mu$  (fig. 13).

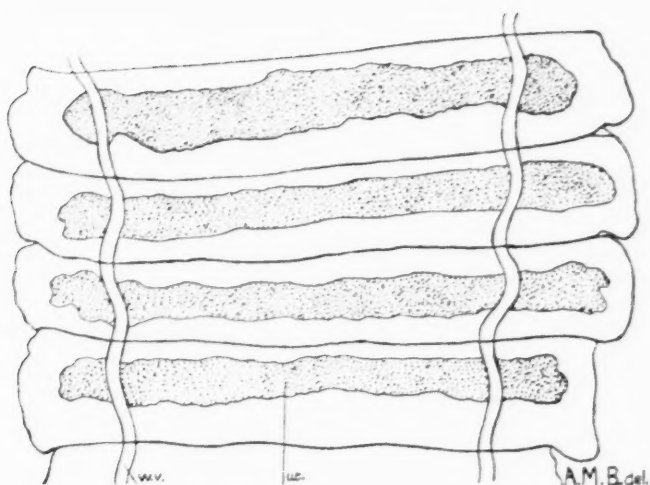


FIG. 13. *Hymenolepis annandalei*, n.sp. Whole segments showing fully developed uterus. ut.—uterus; w.v.—water vessel.  $\times 35$ .

#### DIAGNOSIS

The worm bears a very close resemblance to *H. brasiliense*, Führ. The only difference between them is that in Führmann's species the testes are in a line, whilst in *H. annandalei*, n.sp., this is not the case.

I have pleasure in naming this species in honour of Dr. Nelson Annandale, Director of the Zoological Survey of India.

#### Sub-genus *Echinocotyle*, Blanchard, 1891

##### *Echinocotyle uralensis*, Clerc, 1902

1. One specimen from intestine of snipe. Potsengbam, near Loktak Lake, Manipur, Assam (2600 feet), Station 1. Manipur Survey, 14.2.20.

This specimen agreed with Clerc's description, except that the hooks when isolated measured up to  $74\mu$ . In the type species they measure from  $54\mu$  to  $66\mu$ . In some segments testes were entirely absent; two other segments contained only one in each, and in four or five other segments the posterior aporal testis was absent.

2. One specimen from gut of a snipe. No further data given.

3. One specimen, without head, from *Gallinago* sp. (snipe). Berhampur, Bengal. Collected by Lt.-Col. Clayton Lane, 12.3.12.

4. Two specimens (one with a head) from intestine of a snipe. Berhampur, Bengal. Collected by Lt.-Col. Clayton Lane, (No. 219b), 17.12.12

## Family TAENIIDAE, Ludwig, 1886

*Diploposthe laevis* (Bloch, 1782), Jacobi, 1896

1. One complete specimen from intestine of *Netta rufina* (red-crested pochard). Zoological Gardens, Calcutta. Collected by the author, 29.1.14. The specimen was stained and mounted.

2. Fragments from intestine of *Nyroca ferina*, Chilka Lake, Orissa, India, 24.11.14. Numbered Z.E.V.  $\frac{6874}{7}$  in the collection of the Indian Museum.

*Diploposthe* sp. (? *laevis*)

A fragment from intestine of *Strepsilas interpres* (turnstone plover). Chilka Lake, Orissa, 24.11.14. (Chilka Survey).

## Family ACOLEIDAE, Ransom, 1909

*Dioicocestus novae guineae*, Führmann, 1914

1. Three specimens from intestine of *Podiceps albipennis* (the little grebe). Zoological Gardens, Calcutta. Collected by the author, 1.5.17.

They had the following measurements:—

TABLE III.

Number						Length	Breadth	Thickness
1.	Male	...	...	...	...	10.0 ccms.	5.0 mm.	about 1.0 mm.
2.	Male	...	...	...	...	10.0 cms.	3.5 mm.	about 1.0 mm.
3.	Female	...	...	...	...	17.0 cms.	5.9 mm.	1.6 mm.

The head of the female worm (No. 3) is armed with at least 12 hooks, 320 $\mu$  long (fig. 14). Possibly a few hooks were missing. In shape these



FIG. 14. *Dioicocestus novae guineae*, Führmann. Showing hook.  $\times 80$ .

hooks are similar to those figured by Lühe for *D. aspera* (Mehlis), but in the latter species they measure only 200 $\mu$  to 218 $\mu$ , and are 14 in number. In the male specimens (Nos. 1 and 2) the hooks were missing, but the

impressions made in the parenchyma by these hooks were clearly visible. The only trace of genitalia in these two male strobilae consists of two cirrus pouches in each segment, each of which measures  $750\mu$  in length and  $330\mu$  in breadth. No spines were seen on the cirrus although carefully looked for. In the female strobila the ovary had almost entirely degenerated. There were a number of gravid segments; the eggs measured about  $50\mu$  and the onchosphere  $28\mu$ .

Four species of this genus are now known (Table IV).

TABLE IV.

Species	Locality	Host	MALE		FEMALE		Rostellum	Suckers
			Length	Breadth	Length	Breadth		
<i>trouai</i>	... Argentine	<i>Plegadis guarauna</i>	mm. 70	mm. 4.0	mm. 60	mm. 5.0	Practically absent	Practically absent
<i>mytilus</i>	... Jamaica, Brazil	<i>Podiceps dominicus</i>	45-130	2-2.5	100-190	3.5-4.0	Very small	Very small
<i>aspera</i>	... Europe	<i>Lophaethya cristata</i> and <i>L. griseigena</i>	280	6.0-9.0	340	8.0-11.5	Well developed with 14 hooks 200-218 $\mu$ in length	Well developed
<i>novae guineae</i>	New Guinea	<i>Podiceps novae bellandiae</i>	60	3.5	50	4.5	Moderately developed with 18-20 hooks	Moderately developed

As the Indian specimens have well-developed suckers and a large rostellum, they are closely related to *D. aspera* and *D. novae guineae*. They differ from the former in size and in possessing larger hooks, and agree with Führmann's description of the latter genus. No hooks were present in Führmann's specimens.

2. A second very young female specimen of what I believe to be this species was obtained from the same host and locality, by Dr. Baini Prashad, 2.2.18. The specimen was strongly contracted and was ripe, but no gravid segments were present. It was sectioned and mounted.

*Cestode* sp.

A few fragments from *Sterna fluviatilis*. Zoological Gardens, Calcutta, 3.1.15.

Order *PSEUDOPHYLLIDAE*, Carus, 1863

Family *DIPHYLLOBOTHRIIDAE*, Lühe, 1910

Genus *Ligula*, Bloch, 1782

Bothria as well as external segmentation completely absent from the larvae ; both develop simultaneously with the maturation of the sex-organs in the definitive host, where the external segmentation which does not correspond with the internal is confined to the anterior end. Longitudinal and transverse muscles irregularly interwoven in the anterior end, posteriorly separated into an inner transverse and an outer longitudinal layer.

Type (and only) species : *Ligula intestinalis* (L.).

*Ligula intestinalis* (Linnaeus, 1758)

Three larval forms from the coelome of three specimens of *Danio acquipinnatus* (McClelland), collected by S. L. Hora, Esq., Indian Museum ; Pung-Ka-Mem-John stream, Cherrapunji, Khasi Hills, Assam, 28.10.21, and numbered  $W \frac{423}{1}$  and  $W \frac{424}{1}$  in the collection of the Indian Museum.

Lühe in 1898 arrived at the conclusion that there is only one species of *Ligula*, and this conclusion was accepted by Linstow in 1901 and by Cooper in 1918.

The synonymy of both the larval and adult forms is very extensive, and a complete list is given by Cooper (1918). The larval forms occur in the body cavity of Teleosts and the adults occur in the intestine of wading and diving birds.

Our specimens are typical in every respect and call for no comment.

The author has previously (1913) recorded the occurrence of this larva in the intestine of the following Indian fishes, viz., *Labeo calbasu* and *Nemachilus rupicola*.

Another larval form, viz., *Schistocephalus solidus*, occurs much less commonly in the abdominal cavity (and occasionally in the stomach and intestine) of bony fishes, but the larval form of this species is characterised by the fact that it is definitely segmented, and possesses two bothria, whereas in *Ligula intestinalis* bothria and all traces of external segmentation are absent.



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## A NEW MALARIA PARASITE OF MAN

BY

J. W. W. STEPHENS

*(Received for publication 3 October, 1922)*

## PLATE XVI

During the course of experimental work on the treatment of malaria, carried out at the Liverpool School of Tropical Medicine from 1917 to 1921, it was the practice always to control the clinical results of treatment by microscopical blood examinations.

Occasionally—perhaps some half-dozen times—parasites other than 'ring' forms were found in films, and doubt arose as to whether they were quartan or simple tertian.

The present paper concerns the parasites found in one such case.

Private J.—, 188817.

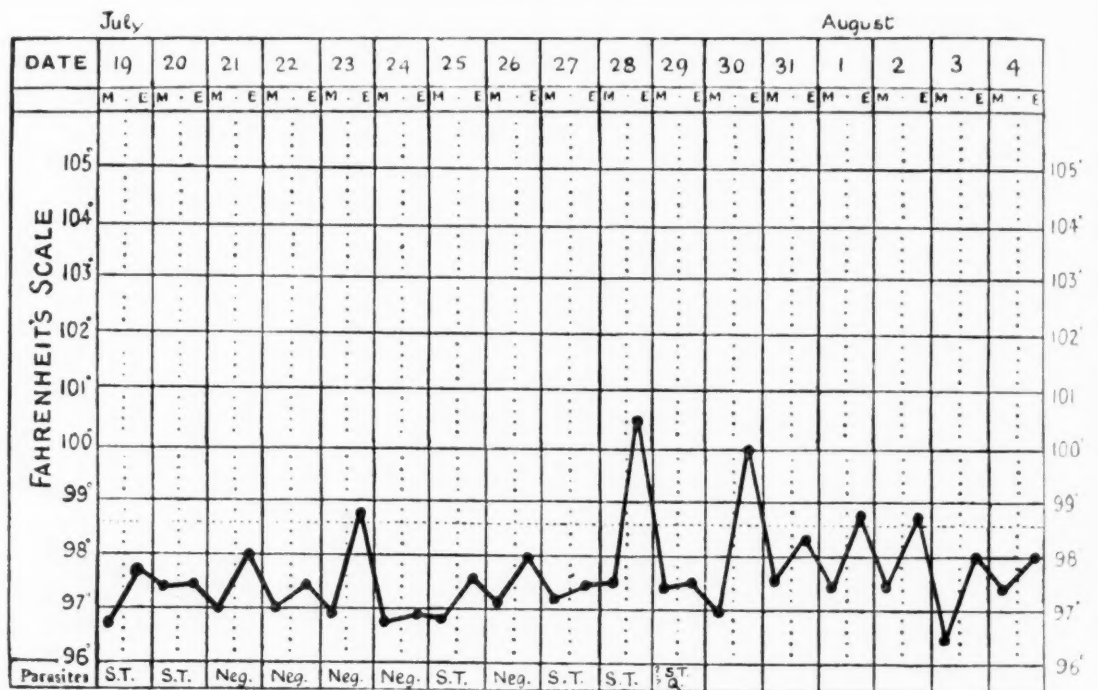
December, 1916	...	...	'Malaria,' East Africa.
January, 1918	...	...	Left East Africa.
8.4.18 to 11.4.18	...	...	The blood films for these dates, made for the purpose of counting the leucocytes, are still in existence and show Simple Tertian parasites.
and			
27.7.18 to 4.5.18			
19.7.18 to 20.7.18	...	...	The films still in existence show Simple Tertian parasites.
21.7.18 to 24.7.18	...	...	The entry made in the Card Index was 'Negative.'
28.7.18	...	...	The entry was '? Simple Tertian.'
29.7.18	...	...	The entry was '? Simple Tertian, ? Quartan.'

A re-examination of these two latter films, still in existence, show peculiar forms.

30.7.18 to 3.8.18	...	...	Owing to the doubt as to the nature of the parasites found, and from the fact that this was not the first time that such doubt had arisen, a series of films approximately at 4-hourly intervals during the daytime was made on the above dates. They were stained for 1 hour with Leishman's stain, and show perfectly well at the present time the characters to be described.
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The Temperature Chart of 28.7.18 to 30.7.18 (fig. 1) shows a tertian periodicity, and the parasite findings for these days are as follows :

28.7.18	9 a.m.	...	...	Young forms absent. Incompletely segmented forms with 6-8 chromatin masses.
29.7.18	...	...	...	Young forms in successive stages of growth during the day.
30.7.18	9 a.m.	...	...	Incompletely segmented forms.
	1 p.m.	...	...	Completely segmented forms and young rings.
1.8.18	2 p.m.	...	...	Completely segmented forms and young rings again present.



The periodicity of the parasite appears therefore to be tertian. Although a cycle of development is passed through from 30.7.18 to 1.8.18 the temperature on the latter date reaches only 98.8° F.

#### THE PARASITE

##### Young forms.

Small 'rings' indistinguishable from 'rings' of other species, or round or oval forms with little or no clear area ('vacuole') around the nucleus. No indication of amoeboid activity as judged by irregularity of form. The red cells in which the parasites occur are not uncommonly oval with irregular margins—*fimbriated*. At this stage the cells are not enlarged and (generally) show no Schüffner's dots.

*Medium-sized forms.*

These are the characteristic forms. They resemble rather closely quartan parasites in the appearance they present of 'solidity' or 'compactness,' and the amount of chromatin and the distribution of the pigment in a lateral band are appearances that recall quartan, but no band-like or 'meridional' forms, as seen in the case of the quartan parasite, were found. They are globular or oval, and occur so frequently in *oval* red cells that it can hardly be a matter of chance but one of actual significance. In forms with one chromatin mass this is often lateral and roughly triangular. There is a complete absence of the irregular, fantastic, 'straggling' parasitic forms occurring in cells of not uncommonly twice the normal diameter so characteristic of simple tertian parasites. Schüffner's dots are now well marked.

*Segmenting forms.*

The gradual transition from young rings to segmenting forms can be traced with ease and certainty. The maximum number of segments (merozoites) appears to be 12. Forms occur with as few as 6 nuclear masses and with the pigment concentrated into a single mass, but it is impossible to be certain in the absence of complete segmentation of the protoplasm whether division is completed. The cell in which these forms lie is either normal in size or slightly enlarged. A slight margin showing Schüffner's dots is often seen, and the cell is clearly decolorized.

The characteristics then of this parasite so far as concerns the medium forms are a non-amoeboid, pigmented, compact, round or oval parasite, resembling quartan, in a red cell showing Schüffner's dots, which is either normal in size or only slightly enlarged. The pigment, so far as can be judged in stained specimens, appears to be brownish black, and granular rather than spicular. A double infection of a red cell was only seen once, viz., with 2 contiguous quarter-grown oval parasites in an oval cell.

No forms that could be interpreted as gametes were seen.

Now and then, but it has been a rare occurrence, I have encountered a form which I could not distinguish from simple tertian.

This parasite appears to resemble that found by Ahmed Emin in 1914 in the case of six pilgrims at Camaran in the Red Sea, and figured and described by him as *Plasmodium vivax*, var. *minuta*. I have been unable



to procure Ahmed Emin's specimens for examination, so cannot come to a decision as to the identity of his parasite with the present one.

The characters of this parasite appear to me to be different from any of the usually accepted species and I propose to call it *Plasmodium ovale*.

#### REFERENCE

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## EXPLANATION OF PLATE XVI

*Plasmodium ovale*, n.sp.  $\times 1800$

Figs. 1—5. 'Ring' forms

Figs. 6—13. Medium forms.

Figs 14—22. Pre-segmenting and segmenting forms.



1



2



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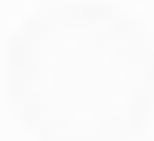
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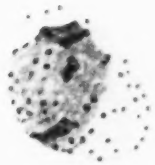
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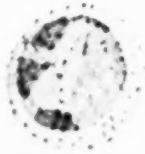
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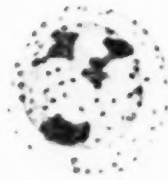
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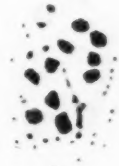
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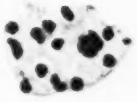
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22





# NOTES ON THE BIONOMICS OF *STEGOMYIA CALOPUS*, MEIGEN, IN BRAZIL

## PART I

BY

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*(Received for publication 21 October, 1922)*

The following work, which was carried out in Manaus, does not attempt to deal comprehensively with the bionomics of *Stegomyia calopus*, but is mainly concerned with various points which attracted attention while breeding these mosquitoes in the laboratory. It was undertaken chiefly owing to the noting of some slight differences in the bionomics of this mosquito, as compared with those observed in certain other countries.

The factors influencing the bionomics of *S. calopus* have been shown to be complex, and, owing to its sensitiveness to environmental conditions, it is doubtful if conclusions can be drawn from the comparison of experiments which have not been carried out under similar conditions of time, place, etc. The effects of many of these factors may be studied by comparison with controls, the only difference between the control and the subject of the experiment being the factor under investigation. Where applicable, this method was adopted in the following experiments.

## METHODS

The adult mosquitoes were kept in wire-gauze cages, and fed on sugar solution. When eggs were required they were allowed to feed on human blood. In the experiments, eggs and larvae were kept in glass jars on a bench in the laboratory, and those used in any one experiment in which a comparison was to be made were kept together, so that the temperature and amount of light reaching each one were the same.

Except where otherwise stated, larvae which hatched out were removed every twenty-four hours. The eggs used in each experiment where comparisons are made were from the same batch of eggs, but not necessarily from the same adult, the eggs being mixed before distribution. They were always less than twenty-four hours old at the beginning of each experiment.

### TEMPERATURE

The temperature range in Manáos throughout the year is small. According to official figures from 1902 to 1914, the average of the annual mean temperature was  $28.2^{\circ}\text{C}.$ , the absolute maximum  $38.6^{\circ}\text{C}.$ , and the absolute minimum  $18.8^{\circ}\text{C}.$  During the experiments the laboratory temperature varied between  $33^{\circ}\text{C}.$  and  $24.5^{\circ}\text{C}.$  The temperature of the water in the jars was always found to be within  $2^{\circ}\text{C}.$  of the atmospheric temperature, and was frequently the same. The daily range of the laboratory temperature was usually less than  $6^{\circ}\text{C}.$

### LAYING OF EGGS

Fielding (1919) and Bacot (1916) found that females preferred contaminated water to clear for laying eggs in. A few experiments were carried out to discover if this preference existed in Manáos.

Watch glasses containing the waters to be compared were placed in the breeding-cage, losses due to evaporation being replaced from time to time. The results, which are given in Table I, are similar to those of Fielding and Bacot.

TABLE I.  
Laying of Eggs in Different Waters.

Duration of Experiment	Contents of Glass	No. of Eggs laid	Percentage of Total
72 days	Tap water ... ..	560	26
	River water ... ..	1590	74
32 days	Tap water ... ..	563	10.6
	River water ... ..	1682	31.5
	Water from cesspool	3092	57.9

Bacot states that it is misleading to say that the eggs are deposited on the surface of water, as in the great majority of cases they are to be found on the wet margins of the receptacle or other object, and that no instance of an egg being laid on a dry surface was observed.

In the following experiment it was found that in captivity the majority of eggs were laid on a damp surface, when available. A watch glass containing rain water and a piece of blotting paper floating on it, the areas of the blotting paper and the water surface exposed being approximately equal, was placed in the breeding-cage. The results are shown in Table II.

TABLE II.

The Numbers of Eggs laid on Water and on a Damp Surface.

Duration of Experiment	Eggs found		
	On Blotting Paper	On Water	On Dry Glass
44 days	1966	568	88

No mosquitoes were observed laying eggs on a dry surface, and those found dry were probably stranded by capillary attraction and left above water level by evaporation. This could not occur with the blotting paper, as it was floating. Fielding's findings in Queensland were similar.

So far as hatching was concerned, no difference was found between those laid on the moist blotting paper and those laid on water.

Wild *Stegomyia* have been observed on several occasions laying eggs in barrels standing in the enclosure behind the laboratory. The eggs were always laid on the wet sides of the barrel just above the water surface. In glass jars placed outside, eggs were laid on the water, usually near the sides, and many adhered to the jar as the water evaporated.

#### HATCHING OF EGGS

Difficulty was at first experienced in getting eggs which were left floating as laid, to hatch. An attempt was, therefore, made to discover the cause of this, and several factors were found to

influence the hatching. Those investigated were, position of the eggs (floating or submerged), presence or absence of disturbance, presence or absence of food, and the nature of the water. Many other factors, such as bacterial action, formalin, temperature, humidity, drying, lysol, petroleum, soft soap emulsion, and soap solution, are stated to influence the hatching of the eggs.

Many experiments were carried out, all of which are not given below, but the following illustrate the points mentioned.

#### HATCHING OF FLOATING AND SUBMERGED EGGS

Fielding (1919) in Queensland, and Bacot (1916) in West Africa, found no difference in the hatching of floating and submerged eggs. In Manáos the difference was definite. Even under the most favourable conditions, floating eggs only occasionally hatched.

A batch of fifty-four eggs laid during the previous twenty-four hours were divided into two equal lots. Each lot was placed in a jar containing tap water (7.4 c.c. per egg), rice was added and the eggs were agitated by stirring daily for one minute. One lot was submerged on the first day, and the other left floating for twenty days. The results are shown in Table III.

TABLE III.

Hatching of Floating and Submerged Eggs.

Eggs floating for 20 days.			27	Eggs submerged on 1st day			27
Hatched by 20th day	...	...	0	Hatched by 20th day	...	...	77%
Hatched after submergence	...	...	96%	Hatched by 39th day	...	...	96%

This result has been confirmed by similar experiments with and without added food, and disturbance, and in rain water, but occasionally a few eggs hatched. Why some eggs hatched when floating, although the majority did not, is not apparent.

Under natural conditions the eggs were observed to be

submerged by various agencies, chief of which appeared to be rain. In Manáos rain falls at all seasons.

Jars containing eggs were exposed to rain with the following results :—

41 eggs out of 56 were submerged by 15 minutes rain.

25 „ „ 26 „ „ „ 5 „ „

18 „ „ 27 „ „ „ 5 „ „

Eggs laid or becoming stranded on the sides of the receptacle became attached when dry, and when the water rose again remained attached and were therefore submerged. Fully developed larvae usually, but not always, submerged floating eggs, seizing the eggs with the mouth, pulling them below the surface and releasing them.

The following results were obtained :—

4 larvae submerged 89 out of 110 eggs in 2 days.

4 „ „ 19 „ „ remaining 21 eggs in 27 days.

6 „ „ 34 „ „ 34 eggs in 24 hours.

4 „ „ 4 „ „ 27 eggs in 11 days.

The reason for the differences shown is not known.

Floating eggs were also found to be submerged by insects falling into the water.

#### EFFECTS OF DISTURBANCE ON HATCHING

Mitchell (1907), in the United States, records that Duprée found agitation to be a great factor in the hatching, and that if left undisturbed eggs may remain unhatched for over a year. Bacot (1916), in West Africa, failed to obtain a decisive result on this question of agitation, and also (1918) casts doubt on the value of Mitchell's records.

In Manáos the majority of eggs did not hatch unless disturbed. It has already been stated that floating eggs did not usually hatch, even when disturbed. When submerged before they were ready to hatch and left undisturbed, they also usually remained unhatched when no food was added. This is shown in Table IV:



TABLE IV.

Effects of Agitation on Hatching in the Absence of Added Food.

Nature of water		Submerged 1st day		Control
		No food added		Food added
		Not Agitated	Agitated	Agitated
Tap water	No. of eggs ... ..	50	30	17
	Hatched in 1 month	2 %	100 %	100 %
Rain water	No. of eggs ... ..	140	30	25
	Hatched in 1 month	0	33 %	96 %

In this experiment two batches of eggs were used, one in tap water and the other in rain water. The control was merely to demonstrate that the eggs were fertile under favourable conditions. The agitation consisted of stirring for one minute daily. The amount of water per egg was the same for each batch.

In the following experiment shown in Table V, some hatching took place in the presence of added food, but less so than among the controls. Rice was added to each jar, and all eggs were submerged on the first day.

TABLE V.

Effects of Agitation on Hatching in the Presence of Added Food.

Water	230 c.c. in each jar	Not Agitated	Agitated
Rain water ... ..	No. of eggs ... ..	30	30
	Hatched in 12 days ...	40 %	100 %

It may be added that a further 36 per cent. hatched when agitation was provided.

Various methods of providing disturbance were tried. The dropping of water into the jar so as to submerge the eggs was usually followed, in the presence of food, by the hatching of the majority when they were four days old. Rain had a similar effect. A jar containing twenty-six eggs, four days old, floating in tap water, was placed in rain for five minutes. Twenty-five eggs

were submerged, and within four hours eighteen larvae were removed. A control showed no hatching.

Stirring or aerating the water for one minute daily, or the addition of one or more larvae, provided an effective stimulus. In the presence of added food, the disturbance caused by larvae appeared to be only slightly more effective than one minute's stirring, as shown in Table VI.

TABLE VI.

Larvae *v.* Stirring in the Hatching of Eggs in Presence of Added Food.

Two fully-grown larvae present					Stirred one minute daily				
No. of eggs	...	...	...	30	No. of eggs	...	...	...	30
100% hatched in	...	...	...	5 days	100% hatched in	...	...	...	8 days

Each jar contained 230 c.c. of rain water. The eggs were submerged at the beginning of the experiment.

In tap water little difference was observed, the larvae hatching the eggs slightly faster. In the absence of added food, however, larvae were more effective, as shown in Table VII.

TABLE VII.

Larvae *v.* Stirring in the Hatching of Eggs in Absence of Added Food.

Two fully-grown larvae present					Stirred for one minute daily				
No. of eggs	...	...	...	30	No. of eggs	...	...	...	30
Hatched in 18 days	...	...	...	76%	Hatched in 18 days	...	...	...	10%
Total hatched after addition of rice	...	...	...	96%	Total hatched after addition of rice	...	...	...	90%

Each jar contained 230 c.c. of tap water. The eggs were submerged at the beginning of the experiment.

A similar result was obtained in rain water. In these experiments fully grown larvae which pupated or died were replaced by others. It seems probable from these and other results that the larvae, possibly through their excretions, had an effect on the eggs similar to that of the addition of food.

## EFFECTS OF FOOD ON HATCHING

In Table VIII it is shown that the addition of rice rendered the conditions more favourable to hatching.

TABLE VIII.

Influence of Addition of Rice on Hatching.

Rice added	...	...	...	...	...	○	50 mgms
No. of eggs in each jar	...	...	...	...	...	30	30
Hatched by 20th day	...	...	...	...	...	33%	90%
Hatched after addition of rice	...	...	...	...	...	83%	—

The eggs were floating till submerged on the fourth day, after which they were stirred daily for one minute. Each jar contained 230 c.c. of rain water.

In other experiments where rice had not at first been used, but other conditions suitable to hatching were present, its addition was invariably followed by hatching, comparison with controls indicating that the hatching was due to the addition of the rice.

## EFFECTS OF DIFFERENCES IN WATER ON HATCHING

Tap water containing more organic matter than rain water might have been expected, on grounds of possible food supply, to be more suitable for the hatching of eggs than rain water. The latter was, however, found to be preferred by the mosquito for laying eggs on, and to be more suitable for the hatching and development of larvae. Tap water consists, in Manáos, of sedimented river water, an analysis of which, made by Mr. W. J. Debdin, F.I.C., F.C.S., has been published by Thomas (1910). According to this analysis the water contained a considerable amount of albumenoid ammonia, apparently derived from vegetable matter, no nitrates, but *B. coli* in 0.1 c.c., and in other ways, resembles what is usually described as a peaty water.

Where conditions were favourable for hatching, all, or nearly all, eggs hatched, whether in tap or rain water. In Table IX are shown the results of an experiment in which the conditions were favourable,

there being 50 mgms. of rice in each jar; the eggs were submerged on the fourth day, and two larvae were added to each jar.

TABLE IX.

Hatching of Eggs in Different Waters under Favourable Conditions.

Nature of water (7 c.c. per larva)					Rain water	Tap water
No. of eggs in each jar	...	...	...	...	30	30
Hatched by 5th day	...	...	...	...	100%	93%

Where conditions were less favourable, eggs hatched more readily in rain water, as shown in Table X. Here no food and no larvae were added. The eggs were floating till submerged on the fourth day.

TABLE X.

Hatching of Eggs in Different Waters under less Favourable Conditions.

Nature of water (4.6 c.c. per larva)					Rain water	Tap water
No. of eggs in each jar	...	...	...	...	50	50
Hatched by 5th day	...	...	...	...	52%	22%

Similar results were obtained under other conditions, and it will be shown subsequently that larvae developed more quickly in rain water.

#### VIABILITY OF EGGS KEPT IN WATER

It is well known that eggs will hatch after being kept dry for many months. Bacot (1916) stated that some eggs when kept continually immersed did not hatch for periods of from two to five months. This was tested, and the results are recorded in Tables XI and XII. The eggs were stored in the water in jars, which were undisturbed as far as possible and to which no food was added. At the end of each month shown, a number of eggs were removed and examined, the split and collapsed ones being rejected and the others submerged in rain water to which rice was added and stirred daily.

TABLE XI.

Viability of Eggs stored in Tap Water.

	Removed after	No. tested	Hatched	Pupated	Adults
Eggs stored floating ...	4 months	20	45 %	0	0
	5 months	40	15 %	0	0
	6, 7, 8 months	160	0	0	0
Eggs stored submerged	4 months	30	46 %	6.6 %	6.6 %
	5 months	30	20 %	0	0
	6 and 7 months	30	0	0	0

TABLE XII.

Viability of Eggs Stored in Rain Water.

	Removed after	No. tested	Hatched	Pupated	Adults
Eggs Stored Floating	3 months	40	92 %	75 %	75 %
	5 months	30	40 %	...	... *
	7 months	25	0	0	0 *
Eggs stored submerged	3 months	40	100 %	95 %	95 %
	4 months	40	95 %	95 %	95 %
	5 months	32	56 %	...	... *
	7 months	15	0	0	0 *

\* These observations were kindly made for me by Dr. R. M. Gordon after my departure from Manáos.

From each of the four batches used in the experiments shown in Tables XI and XII controls were taken and placed under conditions favourable to hatching, and were found to be fertile to the extent of 96 to 100 per cent. adults being eventually produced. Rejections on account of splitting or collapse of the eggs amounted to 7 to 11 per cent. of the eggs in each jar. Of the adults produced, sixty-four were males and forty-four females.

Comparison of the figures in Tables XI and XII would indicate that the eggs retained their viability longer in rain water than in



tap water, but such a comparison is not justifiable as different batches of eggs were used, and the times of the experiments, although overlapping, were not identical.

Eggs were, therefore, found to be able to remain alive for five months in water, either floating or submerged. This accords with Bacot's findings in West Africa. Mitchell (1917) records survival immersed at over a year, but gives no details.

### THE DEVELOPMENT OF LARVAE AND PUPAE

The development of *S. calopus* larvae is influenced by the nature of the water, its amount per larva, the presence of food and its nature, and other factors which were not investigated.

In each of the experiments shown in Tables XIII to XVI the larvae used were hatched from the same batches of eggs during the same respective periods, and were all less than twenty-four hours old at the beginning of the experiments.

#### NATURE OF THE WATER

The only waters compared were tap water and rain water. The result is shown in Table XIII. The larvae hatched in the water in which they were subsequently kept. 0.02 per cent. of rice was added to each jar, and the water was aerated daily for one minute by bubbling air through it.

TABLE XIII.

Development of Larvae in Tap Water and Rain Water.

Water (11 c.c. per larva)	Rain water	Tap water
No. of larvae ... ..	24	24
Pupation commenced ... ..	10th day	22nd day
Percentage giving pupae ... ..	79%	8%
Percentage giving adults ... ..	79%	0
Average larval life of those pupating ... ..	19.9 days	22.5 days

In the tap water all the larvae became fully developed, but were undersized. The mortality was probably associated in some way

with the water, but larvae were quite capable of developing in tap water when less crowded. Fourteen out of fifteen became adults under similar conditions in tap water where the concentration was 50 c.c. per larva.

#### CONCENTRATION OF LARVAE

The effects of overcrowding are shown in the following experiments, and indicate that where experiments are carried out to test the values of different foods or waters, the results are not comparable if the concentration of larvae has not been the same in each experiment.

TABLE XIV.  
Result of Varying the Amount of Tap Water per Larva.

Amount of water per larva	50 c.c.	250 c.c.
No. of larvae in each jar ... ..	15	15
No. of pupae produced ... ..	15	15
No. of adults produced ... ..	14 (9♂♂; 5♀♀)	15 (10♂♂; 5♀♀)
Average duration of larval and pupal stages, ♂♂ ...	14.2 days	10.8 days
Average duration of larval and pupal stages, ♀♀ ...	17.8 days	13.0 days

This experiment is complicated by the fact that in the jar with 50 c.c. of water per larva there was only one-fifth of the quantity of rice present in the other jar (0.006 per cent.). As it became used up, therefore, rice was added gradually to the former jar till equal quantities had been placed in both without raising the percentage present at any time much above 0.006.

TABLE XV.  
Variation of the Amount of Rain Water per Larva.

Amount of water per larva	15 c.c.	30 c.c.
No. of larvae ... ..	20	10
No. of pupae produced ... ..	19	10
No. of adults produced ... ..	19 (12 ♂♂; 7 ♀♀)	10 (5 ♂♂; 5 ♀♀)
Average duration of larval and pupal stages, ♂♂ ...	7.0 days	7.0 days
Average duration of larva and pupal stages, ♀♀ ...	8.3 days	7.4 days

Here the difference is less, possibly owing to more favourable conditions. It was again considered better to provide equal quantities of food per larva in each jar (0.025 per cent. rice and 0.006 per cent. peptone) by gradual addition rather than to commence with a double concentration of food in one jar. The water in each jar was aerated daily for one minute.

Assuming that the method of adding food did not introduce a fallacy, these and other experiments indicate that overcrowding may influence the rate of development.

#### NATURE OF LARVAL FOOD

A large number of organic substances have been found to be suitable as food for the larvae, but some appear to be more so than others. In the following experiment peptone and rice were compared. Two jars, each containing 400 c.c. of tap water, were taken, and rice was added to one and peptone to the other to the amount of 0.012 per cent. on the first and fourth days of the experiment. An equal number of eggs hatched in each jar during the same period of twenty-four hours. The water was aerated for one minute daily. Details are given in Table XVI.

TABLE XVI.  
Peptone v. Rice as a Larval Food.

Food ... ..	Peptone	Rice
No. of larvae ... ..	19	19
No. of pupae produced ... ..	19	19
No. of adults produced ... ..	19 (15 ♂♂; 4 ♀♀)	18 (12 ♂♂; 6 ♀♀)
Average duration of larval and pupal stages, ♂♂ ...	7.1 days	8.6 days
Average duration of larval and pupal stages, ♀♀ ...	8.0 days	9.8 days

Thus under these conditions both male and female larvae develop more rapidly on peptone than rice.

#### DURATION OF LARVAL AND PUPAL STAGES

The duration of the larval stages varied enormously under the conditions described above. The shortest time observed was four days in the case of three male larvae, the food used being peptone

(0.006 per cent.) and rice (0.025 per cent.) in rain water (30 c.c. to each larvae). The longest period recorded was also in the case of a male larva, which did not pupate till the forty-second day after hatching and became an adult two days later; in this case the food was rice alone, and the concentration 9 c.c. of rain water per larva. Macfie (1915) states that under 'normal conditions' the larval stage usually lasts seven to thirteen days, and records an instance where it lasted at least ninety-nine days and produced a healthy adult. Bacot (1916) states that under the most favourable conditions the larval life is passed within four days, but with scarcity of food is prolonged for upwards of seventy days.

Table XVII gives the average duration of a considerable number of larvae living under various artificial conditions in the laboratory.

TABLE XVII  
Duration of Larval Stage.

Sex	No. of larvae	Average number of days	14 days and under
♂	77	9	90.9%
♀	48	14.6	62.5%
Unrecorded	57	7.3	92.9%
Total	182	9.9	84.0%

The duration of the pupal stage did not vary to any great extent. Figures are given in Table XVIII.

TABLE XVIII.  
Duration of Pupal Stage.

Sex	No. of Pupae	1 day	2 days	3 days
♂	96	2	88	6
♀	62	2	53	7
Unrecorded	28	0	17	11
Total	186	4	158	24

Of these figures 85 per cent. took two days, and none took as long as four days. Macfie (1915), in West Africa, records the pupal stage as lasting one to five days, as does Mitchell (1907) in U.S.A.

Table XIX shows the duration of the combined larval and pupal stages.

TABLE XIX.

Duration of Combined Larval and Pupal Stages.

Sex	No. of Larvae	Average number of days	14 days and under
♂	105	10.3	85.7 %
♀	73	14.1	69.8 %
Total	178	11.6	79.2 %

As these records are based on observations made once daily only, and always about the same time, fractions of a day were not recorded. Individuals stated to have taken any particular number of days may be more or less than the number stated by just under twenty-four hours.

#### SURVIVAL OF PUPAE OUT OF WATER

Fielding (1919) placed five pupae on filter paper which was kept moist. All became adults within four days. Of three pupae placed on wet filter paper, allowed to dry and later removed to water, one hatched after thirty-two hours on the filter paper and two failed to hatch after forty-seven and a half and seventy-two hours. Alcock (1921) records that pupae left on the floor of a cage in London hatched after some delay.

In the following experiments the pupae were placed on blotting paper till dry and then transferred to dry glass tubes.



TABLE XX.  
Survival of Dry Pupae.

Duration of pupal stage before drying				Under 24 hours	Over 24 hours, Under 48 hours
No. of pupae	...	...	...	12	3
Result	...	...	...	All died within 48 hours	All hatched within 6 hours

The dry bulb temperature varied between 29° and 25° C. and the wet bulb between 25° and 23° C. during this experiment.

When, however, two lots of dry pupae, four under and four above twenty-four hours, were kept in a dry tube, placed in a stoppered bottle containing water, three hatched out of each lot. It would, therefore, appear that the development of dry pupae was influenced by their age when dried, atmospheric moisture and probably other factors not investigated.

#### MISCELLANEOUS FACTS!

##### RELATIVE NUMBERS OF MALES AND FEMALES

The relative numbers of males and females bred in the laboratory are given in Table XXI.

TABLE XXI.  
Relative Numbers of Males and Females.

Total Adults	No. of Males	Percentage of Total	No. of Females	Percentage of Total
517	323	62.5 %	194	37.5 %

This preponderance of males appeared to be a constant factor under the various conditions employed in the laboratory.

##### REMOVAL OF EGGS BY ANTS

As Bacot (1916) emphasises the fact that ants in West Africa did not carry off eggs, it is worth noting that in Manáos dry eggs were readily attacked by ants which were observed actually removing them, and batches of dry eggs left exposed on the bench overnight were frequently partly or wholly removed by the following morning.

## LAYING OF EGGS WITHOUT FEED OF BLOOD

Fielding (1919) found that eggs were laid when peptone and sugar were given as food without blood. This was tried in Manáos without success, a thick syrup of sugar and peptone being supplied as food in a cage containing about fifty female *S. calopus* and a larger number of males during a period of one month. Eggs were laid when they were allowed to obtain blood. Mitchell (1907) states that *S. calopus* will mate and at times lay without feeding.

## OIL AS A LARVICIDE

Macfie (1917), discussing *S. fasciata* larvae imprisoned beneath a film of oil, describes the efforts made by the larvae to reach the surface. He writes as follows:—'So vigorous does the effort appear to be that it seems not improbable that it would eventually break through a thin film of oil.' The latter occurrence was observed in Manáos in the case of larvae breeding naturally in a barrel of water, the surface of which was covered with a thin film of oil. The larvae on ascending for air did not usually succeed in penetrating the surface film at the first attempt, but only after repeated efforts at different parts of the surface. The larvae from this barrel were the largest observed, but of some removed to the laboratory only a few reached the adult stage, the majority dying owing to failure to get clear of their larval or pupal skins. The pupae and adults which developed were also unusually large.

## SUMMARY

Various points in the bionomics of *S. calopus* in Manáos have been investigated, and in certain minor respects they differed from those described in West Africa and Queensland. Bacot (1918), in discussing the duration of viability of the eggs of *S. fasciata*, writes, 'It seems to me possible that the African and American races of *S. fasciata*—to suggest no smaller division—may differ considerably in constitution.' It is, however, possible that the differences noted in various countries are due to differences in the conditions under which the experiments were carried out, and, therefore, conclusions cannot be drawn from their comparison.

The following facts were found to apply to *S. calopus* under the particular conditions described above:—

1. The adults laid more eggs in cesspit water than in river or tap water.
2. More eggs were laid on a damp surface than on the water surface.
3. Eggs did not usually hatch when floating.
4. Floating eggs were submerged by rain and *S. calopus* larvae.
5. Adults laid more eggs, eggs hatched earlier and in larger numbers, and larvae developed more rapidly and showed less mortality in rain water than in tap water.
6. Some eggs stored in water, floating and submerged, hatched after five months. None hatched after seven months.
7. Larvae developed more rapidly and showed less mortality with peptone as food than with rice.
8. The larval stage varied from four to forty-two days and the average duration in one hundred and eighty-two larvae was approximately ten days, the average for the females being longer than for the males.
10. The duration of the pupal stage varied from one to three days, 85 per cent. of one hundred and eighty-six pupae taking two days.
11. Pupae dried at least twenty-four hours after pupation developed into adults, although kept dry.
12. Throughout the experiments more males than females were produced.
13. Dry eggs were removed by ants.
14. No eggs were laid by adults fed on peptone and sugar only.
15. It was observed that larvae were capable of obtaining air through a thin film of surface oil.

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# THE OCCURRENCE OF THE LARVAE OF *ONCHOCERCA VOLVULUS* (LEUCKART, 1893), IN THE SKIN OF NATIVES OF THE GOLD COAST

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## PLATES XVII AND XVIII

The presence of larvae of *O. volvulus* in the skin has been described by Montpellier and Lacroix (1920, 1921) and by Ouzilleau, Laigret and Lefrou (1921). The former authors stated that the larvae caused itching, resulting in the development of an eruption with papules, vesicles and pustules, which they called filarial itch. The latter authors, on the other hand, considered that the larvae produced an inflammatory reaction in the skin, not especially associated with itching, but giving rise to pseudo-ichthyosis, elephantiasis of the skin of the genital organs and of other parts, leucoderma and atrophy. Brumpt (1920) was not satisfied that the larvae, found in the skin by Montpellier and Lacroix, were those of *O. volvulus*. The observations of Ringenbach and Guyomarc'h (1914), Dubois (1916) and Clapier (1917) are not all in agreement regarding the association or otherwise of elephantiasis with infection with *O. volvulus*.

Larvae of *O. volvulus* were found in the lymph glands by Ouzilleau (1913) and by Fülleborn and Simon (1913). They have since been observed by several others.

In the blood they have very rarely been found. Numerous examinations have been made by Ouzilleau, Rodenwaldt, Rodhain and Van den Branden, Clapier and Montpellier, Lacroix and Boutin, with almost completely negative results. Rodhain and Van den Branden state that Brumpt, in 1904, found them very rarely, that Rodenwaldt found them once among many negative

examinations, and that Ouzilleau found them once only in two thousand examinations. Simon (*loc. cit.*) found that if he squeezed the finger powerfully in making blood smears, larvae of *O. volvulus* were present, and suggested that they were in the lymph that exuded as a result of the squeezing of the tissues.

The following observations were made on prisoners at Secondee, Gold Coast. An inspection of two hundred and ninety men was made in order to note the presence of subcutaneous tumours and also of abnormal conditions of the skin, especially a dry, glistening, wrinkled appearance, with exaggeration of the normal pattern of lines and intervening areas, the condition termed lichenification. Elephantiasis and pronounced thickening of the skin, 'craw-craw' and signs of scratching were also noted. The results are shown in Table I; in the table, L. = lichenification, E. = elephantiasis, T. = thickening of the skin approaching elephantiasis in degree but not specially localised, C.-C. = craw-craw.

TABLE I.

Number examined	Subcutaneous tumours present	ABNORMAL SKIN CONDITIONS				
		L.	E.	T.	C.-C.	Scratches present
290	16	24	1	3	5	20

Twenty-four cases were selected for investigation; of these thirteen had subcutaneous tumours, and in fifteen the skin showed lichenification. Three of the five cases of craw-craw, the three cases of greatly thickened skin, and the single case of definite elephantiasis of the external genital organs, were also included.

#### METHOD OF EXAMINATION FOR LARVAE IN THE SKIN

A piece of skin about half a square centimetre in area was excised from the left lower dorsal region in each case and put into a small tube containing normal (0.85 per cent.) salt solution. A bit of the skin was teased and examined soon after excision, the rest being left in the salt solution for a few hours to allow some of the larvae to escape from the skin. The piece of skin was then removed



from the salt solution to 70 per cent. alcohol for subsequent section. The whole of the deposit that formed at the bottom of the tube was put on a slide and examined for the presence of larvae, then fixed by the addition of two or three drops of Ruge's solution (formalin 2 per cent. containing 1 per cent. acetic acid), dried and stained with warm haemalum solution. Drawings and measurements of the larvae were made with the aid of a camera lucida. The chief clinical features and the results of the examination are summarised in Table II, which shows the occurrence in association of subcutaneous tumours, various clinical skin conditions and the larvae of *O. volvulus* in the skin. The same letters as in Table I are used to denote the skin condition.

TABLE II.

Number of cases	Tumours present	SKIN CONDITION						Larvae of <i>O. volvulus</i> in skin
		Normal	L.	L. & E.	L. & C-C.	T.	C-C.	
7	7	...	4	...	2	1	...	7
3	3	3	...	...	...	...	...	3
5	...	...	1	1	...	2	1	5
1	1*	...	1	...	...	...	...	...
2	2†	2	...	...	...	...	...	...
6	...	...	6	...	...	...	...	...
24	13	5	12	1	2	3	1	15

\* Aspiration of the tumour failed to show larvae of *O. volvulus*.

† Tumours excised and found not to contain *O. volvulus*.

#### EXAMINATION OF THE BLOOD

In each case a thick blood smear was taken from the finger both by day and by night. In fourteen of the cases a more thorough examination was also made; six or more fresh blood preparations from the finger and from the back near the place from which the excised piece of skin had been taken were examined. The skin was strongly squeezed also in order to see whether larvae of *O. volvulus* would be readily squeezed out in this way in cases where they were

known to be present in the skin. In three cases about 3 cubic centimetres of blood were withdrawn from a vein and centrifuged, and the deposit examined. Larvae of *O. volvulus* were found in one case only, Case 6, in blood from the skin of the back. The skin in this case was heavily infected, the excised piece yielding about eight hundred larvae to the saline solution in which it was put. This case was one of the three whose blood was centrifuged. In the three cases with greatly thickened skin, and in the case of elephantiasis, thick smears of blood were taken at night from the finger. Embryos of *Filaria bancrofti*, Cobbold (1877), were found in the case of elephantiasis. In the course of the examinations of the blood and of the excised pieces of skin, larvae closely resembling and probably identical with that of *Acanthocheilonema perstans* (Manson), 1891, were seen in twelve cases, and embryos of *F. bancrofti* in three cases.

#### VITALITY OF LARVAE OF *O. VOLVULUS*

(a) *In normal (0.85 per cent.) salt solution.* The larvae deposited in the tubes of salt solution, when examined a few hours after excision of the pieces of skin, were living and showed active movement. In cover-glass preparations of teased skin, kept in a moist chamber at room temperature (about 25° C.), the larvae were seen to be alive eight hours after removal from the body; on the following day all were motionless. In a case not included in this series, actively moving larvae of *O. volvulus* were found in the skin twenty-two hours after the death of the patient from pulmonary tuberculosis. Larvae obtained by aspiration of a tumour in Case 24 and mounted in salt solution under a cover-glass, ringed with vaseline, showed movements for forty-eight hours.

(b) *In blood.* A drop of blood from the skin of the back of Case 6, containing a few larvae of *O. volvulus*, was covered and ringed with vaseline and kept in a moist chamber at room temperature (25° C.). The larvae showed fairly active movement for over five days. Staining subsequently with haemalum confirmed the identity of the larvae. It is interesting to note that Robles (1919) found that the larvae of *Onchocerca caecutiens*, Brumpt, 1919, rapidly died in blood.

# IDENTITY OF THE LARVAE IN THE SKIN WITH THE LARVAE OF *O. VOLVULUS*

(a) *Morphology.* In the few measurements made of living larvae from the skin, the length varied from 290 to 340 $\mu$ , the breadth from 6 to 7 $\mu$ . Stained preparations showed that they were sheathless, with the cuticle transversely striated for the whole length. The anterior end was free of nuclei for a distance usually of about 10 $\mu$ ; the posterior end was sharply pointed, curved generally at a wide angle, and was free of nuclei for usually the terminal 12 to 15 $\mu$ . The nuclei were small, mostly oval, longitudinally arranged and closely crowded together, the terminal one being usually distinctly elongated. Of the fixed points of Fülleborn's scheme, the 'nerve ring' and last nucleus were the most easily seen and measured. The G1 cell could not be definitely distinguished in many specimens; in those measured the most frequent position was between 69 and 70 per cent. of the length from the anterior end. Some of these features are shown in Table III, and Tables IV and V give comparative measurements of larvae from tumours.

TABLE III.

Films from the deposit in the tubes containing pieces of skin in salt solution; measurements of 168 specimens, 12 from each of 14 cases.

Length		Relative position of 'nerve ring'		Relative position of last nucleus	
Microns	Number	Percentage	Number	Percentages	Number
235	3	21	3	92.5	2
250	16	22	26	93.5	11
265	46	23	82	94.5	72
280	66	24	52	95.5	78
295	26	25	5	96.5	5
Anterior end free from nuclei			Posterior end free from nuclei		
Microns	Number		Microns	Number	
6-8	16		7-11	30	
9-12	148		12-16	133	
13-16	4		17-21	5	

TABLE IV.

Measurements of 50 larvae from a tumour excised from Case 22.

Length		Relative position of 'nerve ring'		Relative position of last nucleus	
Microns	Number	Percentage	Number	Percentage	Number
225	6	21	1	92.5	1
235	19	22	9	93.5	5
250	17	23	20	94.5	35
265	5	24	15	95.5	8
280	1	25	2	96.5	1
295	1	26	1	...	...
310	...	27-29	...	...	...
325	1	30	1	...	...
...	...	31	...	...	...
...	...	32	1	...	...

Anterior end free from nuclei		Posterior end free from nuclei	
Microns	Number	Microns	Number
6-7	10	8-10	9
8-9	32	11-13	30
10-11	8	14-17	11

TABLE V.

Measurements of 40 larvae from fluid obtained by aspiration of tumours in Cases 2 and 7, 20 from each.

Relative position of 'nerve ring'		Relative position of last nucleus	
Percentage	Number	Percentage	Number
21	1	93.5	10
22	6	94.5	22
23	17	95.5	8
24	13	...	...
25	3	...	...

(b) *Relationship to tumours.* In Table II it is seen that larvae were found in the skin in ten of the thirteen cases with subcutaneous tumours. Of the three cases where tumours were present and larvae of *O. volvulus* were not found in the skin, tumours were excised in two and found not to contain *O. volvulus*; in the third case aspiration of the tumour failed to show the presence of larvae. Hence larvae of *O. volvulus* were present in the skin in at least 90 per cent., and possibly in all of the cases with subcutaneous tumours which might be tumours of *O. volvulus*. By excision in Case 22 and by aspiration in Cases 2, 6, 7 and 24, the tumours were shown to contain *O. volvulus*; in these cases larvae were found in the skin. In five cases larvae were present in the skin, but no tumours could be found.

These results confirm the observations of Montpellier and Lacroix and of Ouzilleau, Laigret and Lefrou, that the larvae found in the skin are larvae of *O. volvulus*.

#### SECTIONS OF SKIN

Larvae of *O. volvulus* were seen in sections of the skin in the papillary and sub-papillary layers at all levels. In many sections they could be clearly seen to be quite apart from the blood vessels. No very marked changes in the skin were observed; there was an excess of cells in the papillary layer and around the capillaries. Otherwise the sections appeared to show little departure from normal.

#### THE SKIN CONDITIONS AND THEIR RELATIONSHIP TO THE LARVAE

Lichenification was most evident on the back, buttocks and posterior aspect of the thighs; the shoulders and arms were less affected, the chest and abdomen usually still less, and the face, throat and limb flexures hardly at all (Plates XVII and XVIII).

Sweating of the skin was tested in six of the cases; they were set to do hard work in the sun for a few minutes, and in each case sweating of the affected areas was observed.

Itching does not appear to have been severe in most of the cases. One man whose back showed pronounced wrinkling of the skin,



maintained that there was no itching and his skin showed no marks of scratching, yet the excised piece of skin yielded about five hundred larvae to the salt solution in which it was placed.

The number of larvae counted in the smears of the deposit in the tubes of salt solution varied greatly, the greatest number being seven hundred and eighty-five and the smallest two. In Cases 1 and 12 (Plates XVII and XVIII) the numbers were respectively one hundred and seventy-two and five hundred and forty-three. No relationship between these numbers and the degree of skin affection was established. There were six cases with well marked lichenification in whom the excised piece of skin showed no larvae of *O. volvulus*. On the other hand, in three cases with larvae in the skin the latter presented a normal appearance. Larvae were present in the skin in the three cases of 'craw-craw,' and in the four cases with greatly thickened skin, including the case of definite elephantiasis.

In these various conditions there appears to be nothing to indicate a connection between the presence of the larvae of *O. volvulus* in the skin and the appearances observed.

### CONCLUSIONS

The following conclusions are drawn:—

1. The larvae in the skin were those of *O. volvulus*.
2. They are present in the skin in all, or nearly all, cases with tumours of *O. volvulus*.
3. No clear causal relationship between the larvae and the conditions of 'craw-craw,' elephantiasis and lichenification was shown in these cases.

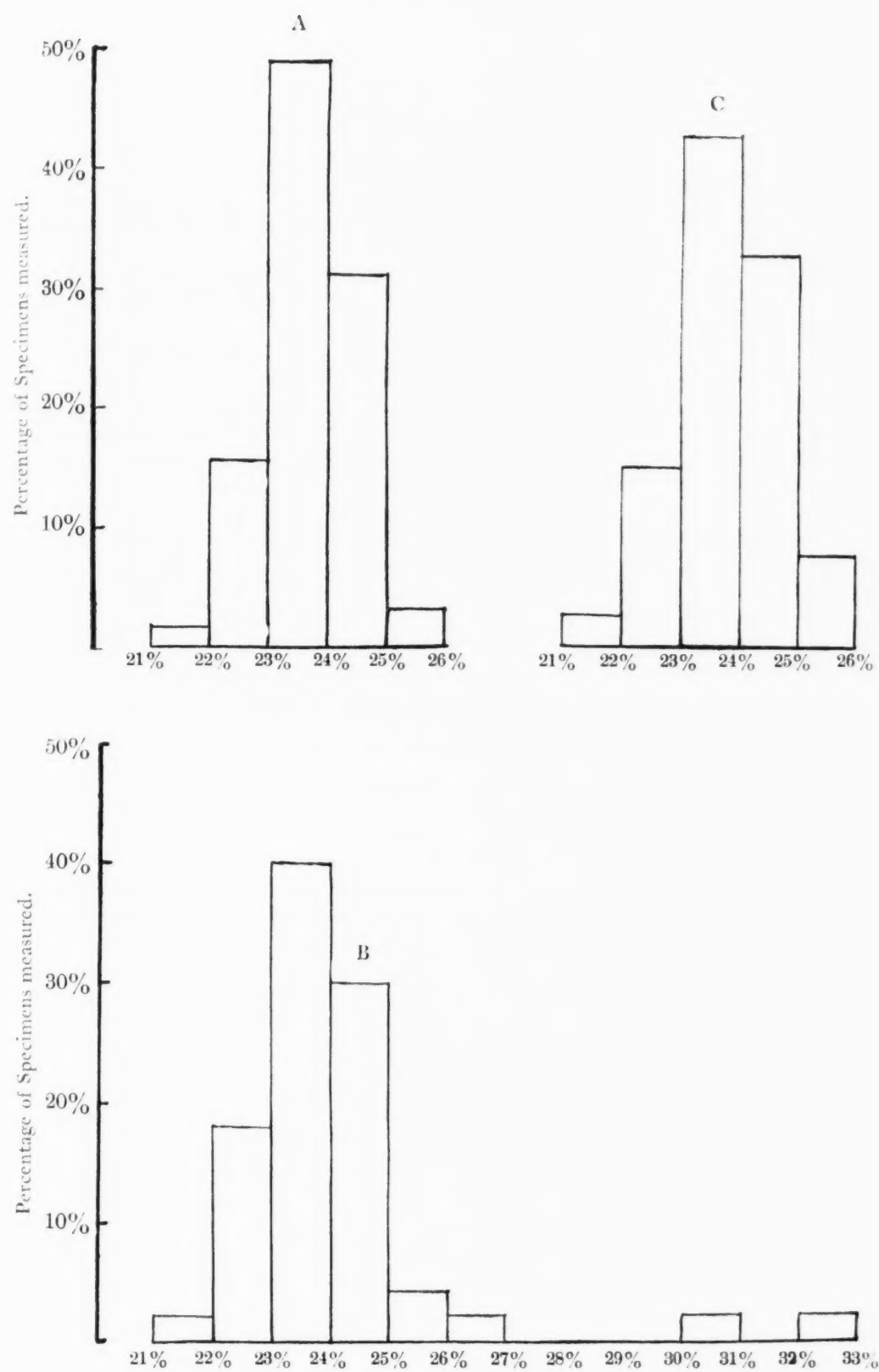


FIG. 1. Comparison of relative position of 'nerve ring' in larvae from (A) skin, (B) excised tumour, and (C) fluid aspirated from tumours; from Tables III, IV and V respectively.

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## EXPLANATION OF PLATE XVII

- Case 1. Portion of back, showing lichenification of the skin. Three small tumours were present: one behind the great trochanter and two over the lumbar vertebral spines. Larvae of *O. volvulus* were present in the skin.





## EXPLANATION OF PLATE XVIII

Case 12. Showing lichenification of the skin. No tumours were found. Larvae of *O. volvulus* were present in the skin. This case had elephantiasis of the external genital organs; the blood contained embryos of *F. bancrofti* and of *A. perstans*.





# A CASE OF SLEEPING SICKNESS (*T. GAMBIENSE*) TREATED BY 'BAYER 205'

BY

J. W. W. STEPHENS,

AND

W. YORKE.

*(Received for publication 20 November, 1922)*H.L.S. *Aet.* 25.

No history of having been bitten by a tsetse fly, but in August, 1921, was for 2½ days in a tsetse belt at Wamba, South of Jemaa, N. Nigeria.

21.11.21. Took to bed with fever at Jemaa. Ill for 8 days, then recovered somewhat, but still unwell and had aching in legs.

—1.22. Glands in neck found to be enlarged, and a blood film was reported to contain trypanosomes. Was sent home.

9.3.22. On examination at the Liverpool School of Tropical Medicine the condition was as follows:—

Lymphatic glands behind the sterno-mastoid on both sides as large as marbles; axillary glands about the same size; inguinal glands (?) enlarged. A circinate rash over the back; over a V-shaped area on the chest, corresponding to the opening in the shirt, deep erythema, with some indication of pitting, probably due to sunburn. Pulse 112. Spleen not enlarged.

## *Blood examination.*

Fresh films negative. Centrifuged blood, trypanosomes found, 1 to 10 fields.

## *Gland puncture.*

A few trypanosomes found.

## *Animal inoculations.*

1. Of 2 mice inoculated intraperitoneally with 0.4 c.c. of citrated blood, one became infected 29.3.22 and died 22.9.22; the other did not become infected.



2. A mouse inoculated intraperitoneally with a suspension of trypanosomes obtained by centrifuging 10 c.c. of the patient's blood showed trypanosomes 17.3.22. The animal is still alive and infected 6.11.22.

3. Various other mice were sub-inoculated from these and became infected. Most of the animals are still alive after six months, and occasionally show trypanosomes in their blood.

4. The trypanosome shows the morphological characters of *T. gambiense*.

#### *Treatment.*

10.3.22. *Atoxyl*, 0.45 gramme, subcutaneously.

13.3.22. *Atoxyl*, 0.45 gramme, subcutaneously.

17.3.22. *Novarsenobillon*, 0.6 gramme, intravenously.

20.3.22. Glands smaller, one on right side as big as large pea. Weight, 124 lbs.

20.3.22. *Novarsenobillon*, 0.9 gramme intravenously.

23.3.22. Patient ill, temperature 103°, blood negative.

27.3.22. Patient better, blood negative, auto-agglutination distinct.

1.4.22. Patient feels well, glands greatly decreased, blood negative, very little auto-agglutination. Weight, 125½ lbs.

8.4.22. Glands hardly palpable, pulse 130, blood negative.

12.4.22. Pulse 108, blood negative, auto-agglutination distinct. Weight, 124 lbs.

18.4.22. Pulse 112, blood negative. Weight, 126½ lbs.

24.4.22. Glands doubtfully palpable, blood negative. Weight, 127¾ lbs.

29.4.22. Pulse 120, blood negative. Weight, 132 lbs.

6.5.22. Pulse 96, blood negative. Weight, 136½ lbs.

14.5.22. Temperature 103°, blood negative. Weight, 137 lbs.

19.5.22. Temperature 100°.

26.5.22. Thick blood film negative. Weight, 137 lbs.

27.5.22. Temperature 99°.

28.5.22. Temperature 101.2°. Thick blood film (stained). 2 trypanosomes found.

30.5.22. Temperature normal. 'Bayer 205,' 0.5 gramme intravenously. Patient vomited a minute or two after the injection.

31.5.22. Temperature subnormal. Pulse 96. Urine no albumen.

- 1.6.22. Temperature subnormal. Pulse 78. 'Bayer 205,' 1.0 gramme intravenously.
- 3.6.22. Temperature subnormal. Pulse 92, a macular rash external to each mamma. 'Bayer 205,' 1.5 gramme intravenously.
- 8.6.22. Urine slightly turbid, no albumen.
- 27.6.22. Temperature normal. Urine slightly turbid, no albumen. 'Bayer 205,' 1.0 gramme intravenously.
- 20.11.22. Patient states that he has remained quite well without any rise of temperature since 27.6.22. Weight, 142 lbs. On examination: an acne-like rash over the back and sternum. Glands in neck not enlarged, but some just appreciable to palpation. Pulse 84-86, a little irregular. Respirations 17. Urine, no albumen. Centrifuged blood (5 c.c.) negative microscopically.

#### SUMMARY

The patient was presumably infected in Northern Nigeria in August, 1921, and had no treatment prior to his arrival in England in March, 1922, although trypanosomes had been found in his blood in January. When first seen in Liverpool on 9 March, trypanosomes were found both in the blood and gland juice. He was given subcutaneous injections of 0.45 gramme Atoxyl on 10 and 13 March, and intravenous injections of Novarsenobillon 0.6 gramme and 0.9 gramme on 17 and 20 March, respectively. These injections were attended by considerable rises of temperature which lasted up to 23 March.

As a result of this treatment the general condition of the patient rapidly improved, the rashes disappeared, the enlargement of the lymphatic glands almost completely subsided, the weight steadily increased, and trypanosomes could no longer be found in the blood. The pulse, however, remained frequent. Except for two rises to 100° F. on 27 March and 9 April, the temperature remained normal until 14 May, when it rose to 103° F. Frequent examinations of the blood during this period were negative. On 28 May the temperature rose to 101.2° F., and trypanosomes were found in the blood. On 30 May an intravenous injection of 'Bayer 205,' 0.5 gramme was given, a second injection of 1 gramme on 1 June and a third of 1.5 gramme on 3 June: the temperature fell to normal after the

first injection, and has since remained normal. The blood was negative on 31 May and also on 3, 8, and 27 June, and the general condition of the patient remained good. No albuminuria developed. On 27 June an intravenous injection of 'Bayer 205,' 1 gramme was given as a 'prophylactic' measure, and since then he has remained in good health.

We are indebted to Messrs. Friedr. Bayer & Co., Elberfeld, for kindly supplying us with a quantity of 'Bayer 205.'

#### NOTE.

In a previous paper by Yorke (1921), details are given of the treatment by 'Bayer 205' in July, 1921, of a case of Rhodesian sleeping sickness. The patient, who is now back in Rhodesia, has remained in excellent health up to the present time.

#### REFERENCE

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# NOTES ON THE BIONOMICS OF *STEGOMYIA CALOPUS*, MEIGEN, IN BRAZIL

## PART II

BY

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*(Received for publication 9 November, 1922)*

### THE ABSENCE OF *STEGOMYIA CALOPUS* LARVAE FROM NATURAL WATERS

From December, 1920, to February, 1922, a fairly extensive search was made in the town of Manáos, its native suburbs, and the sparsely inhabited forest surrounding it, for the breeding-places of various mosquitoes. During this examination *Stegomyia calopus* larvae were never found, except in domestic waters in the immediate vicinity of a human habitation. Young (1921), writing from Manáos, has drawn attention to the same point; Howard, Dyar and Knab (1912) state: 'The larvae are found practically exclusively in artificial receptacles about human habitations. It may be said that the larvae of *calopus* are never found in swamps, in pools or in temporary puddles, even when these are in close proximity to houses.'

The three experiments that follow were devised to test whether the absence of larvae from such waters was due to the disinclination of females to oviposit in them, or to the inability of the larvae to develop when placed there.

Some small stagnant pools situated on the outskirts of the town, about fifty yards from six native houses and about the same distance from the tram line, were selected for the experiments; water from pools of this description will be referred to in the text as 'natural water,' in

contra-distinction to the term 'domestic water' as applied to water in rain barrels, water troughs, etc.

*Experiment I.* A varying number (4 to 11) of *S. calopus* females were confined in two breeding cages, and in each cage were placed six large watch glasses containing water from various sources, both natural and domestic; the position of these glasses was constantly varied to prevent any undue influence of light or shade. Males were introduced and the supply kept constant. Sugar solution was supplied for the males; the females were fed on human blood, a feed being usually offered every other day. The resultant eggs were removed and counted every twenty-four hours. The experiment was continued for five weeks, with the results recorded below.

TABLE I.

	Nature of water supplied	Total number of eggs deposited	Percentage deposited in each type of water
1	Distilled water ... ..	146	9.6
2	Barrel water in which wild <i>Stegomyia</i> were freely breeding ... ..	88	5.8
3	Water from a small pool on the outskirts of the town; this pool harboured <i>Culex</i> and dragon-fly larvae ...	149	9.8
4	Deep pool near (3); contained <i>Culex</i> but no dragon-fly larvae ... ..	309	20.4
5	Same as (4), but algae added ... ..	728	48.1
6	Small pool, same source as (3) and (4). <i>Culex</i> and dragon-fly larvae, but no vegetation ... ..	92	6.0

CONCLUSION. *Stegomyia* in captivity will oviposit as readily in natural as domestic waters. This conclusion agrees with that of Bacot (1916), and of Fielding (1919), but both these authors used domestic water throughout their experiments, and to this added various organic substances.

*Experiment II.* To ascertain whether *Stegomyia* ova and larvae can develop in natural waters, when these have been cleared of inhabitants inimical to the life of the larvae. Eight jars were used; six of these contained 400 c.c. of water and two 800 c.c., all the waters being carefully strained through fine wire gauze, before the introduction of the larvae.



TABLE II.

No. of Jar	Nature of the water used	No. of Ova added	No. of larvae which hatched	No. of Imagoes	Percentage of Ova which completed cycle	Average time taken to complete cycle
1	Tap water, plus 2 grs. of rice ...	24	23	8	33	days 28
2	Water from a barrel in which <i>Stegomyia</i> were freely breeding ...	24	24	10	41	24
3	Small pool natural water which contained dragon-fly and water-beetle larvae ... ..	30	21	3	10	22
4	Small pool natural water which contained no insect life ... ..	30	26	25	83	21
5	Tap water plus 2 grs. of rice ...	30	22	3	10	37
6	Small pool natural water which contained dragon-fly and <i>Culex</i> larvae ... ..	30	25	23	70	25
7	800 c.c. water as in Jar 2 ... ..	30	28	11	36	17
8	800 c.c. water as in Jar 3, plus well-washed duck weed ... ..	30	?	13	43	10

CONCLUSION. *Stegomyia* ova hatch and the larvae develop freely in natural waters after these have been freed from insects inimical to their development.

*Experiment III.* To ascertain whether *Stegomyia* larvae can develop in pools of natural water when (1) unprotected from their insect enemies, (2) protected from their insect enemies.

A small pool such as is described under Experiment I was selected. A careful netting of the pool showed the following inhabitants:—dragon-fly larvae, tadpoles, a few water bugs (*Zaitha* sp.), a small water beetle (previously shown to be harmless to mosquito larvae). No culicidae larvae were found, though the neighbouring pools showed large numbers, mostly *Culex fatigans*.

Into this pool were introduced 900 dried eggs and a few fresh eggs of *Stegomyia calopus* (average fertility of dried eggs was found to be about 40 per cent.), also 300 larvae of *Stegomyia* and 200 larvae of *C. fatigans*, the larvae being on an average 48 hours old. Two glass cylinders,

arranged as shown in fig. 1, were fixed to pointed sticks and these sunk into the mud at the bottom of the pool, about three inches of the cylinder being left projecting above the surface of the water. Into one tube were introduced 50 dried eggs, and 24 fresh eggs of *Stegomyia*. Into the other were placed 20 *C. fatigans* larvae (not more than 24 hours old). During

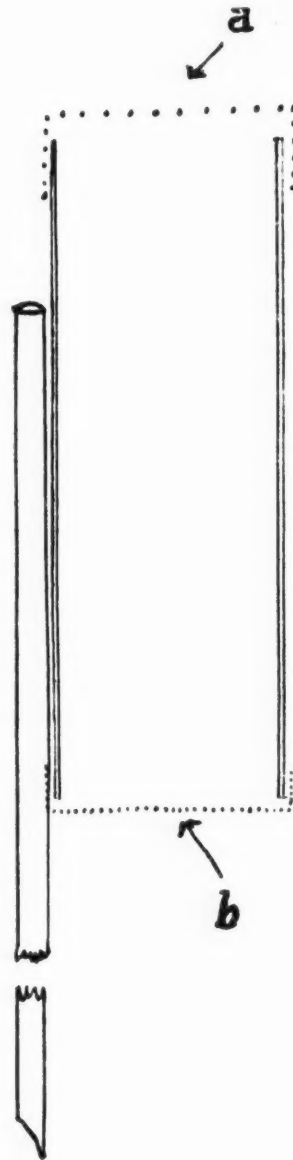


FIG. 1

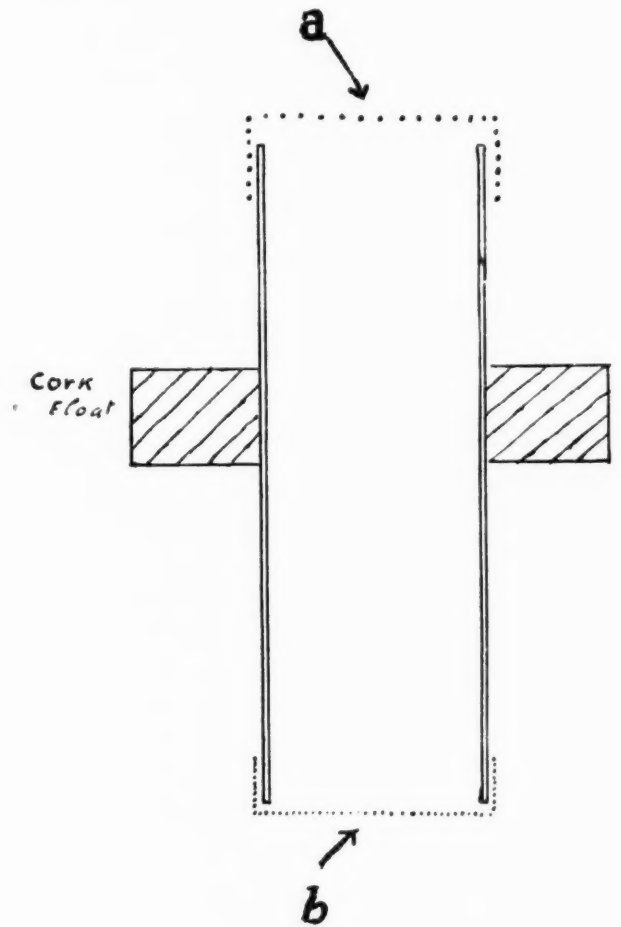


FIG. 2

FIGS. 1 and 2. Glass cylinders, 8 in.  $\times$  2 in., used in Experiment III.  
(a = Mosquito-proof gauze; b = Fine wire gauze.)

the course of the next seven days the pool was regularly visited, but, unfortunately, a week's heavy rain interfered with the observations and the experiment was brought to a close on the eighth day by the rising water in the pool completely submerging the tubes.

The following results were obtained. In spite of very careful searching no *Stegomyia* larvae were found in the pool during the eight days it was kept under observation, the first search being made twenty-four hours after the introduction of the ova and larvae; *C. fatigans* were present during the whole experiment, although on two occasions no larvae could be found; this was probably due to the muddy condition of the water. Of the larvae in the guarded cylinders, both lots appeared to be doing well, three *Stegomyia* imagoes emerging during the eight days; many of the *C. fatigans* larvae reached the fourth stage, but none pupated.

A few months later the experiment was repeated with the following modifications:—600 *calopus* and 255 *fatigans* larvae at all stages of development were added to the pool, and in the cylinders were placed respectively, 26 *calopus* ova (average fertility of a sample found to be 90 per cent.) and 26 *fatigans* ova (average fertility 100 per cent). In lieu of fixing the cylinders to sticks they were attached to cork floats (fig. 2) and allowed to float clear in the pool. The results obtained were precisely similar to those in the previous experiment, except that no imagoes were obtained though both tubes contained apparently healthy larvae. As before, *calopus* larvae disappeared after the first twenty-four hours, while *fatigans* persisted. The observations were brought to a close on the seventh day by the drying of the pool.

SUMMARY. *Stegomyia calopus* ova and larvae, introduced into a natural pool infested with insect enemies, disappeared after the first twenty-four hours, whereas *Culex fatigans* larvae, added under the same conditions, persisted for at least eight days. *S. calopus* ova placed in the same pool, but under conditions protecting them from insect enemies, developed and produced imagoes.

#### ORDER OF HATCHING OF MALES AND FEMALES

Rees (1901) states: 'When mosquitoes are bred in captivity the males as a rule hatch out first, and in greater numbers than the females.' Nuttall and Shipley (1901) comment on this statement as follows:— 'We have found no similar statement elsewhere, and the observations we have made do not tend to confirm his observation. The proportion of males to females has always appeared to us to be fairly equal, and we have counted the sexes on several occasions.' Bacot's (1916) observations in West Africa would appear to confirm Rees. Writing of *Stegomyia* he says:

'... the early males being usually a day quicker in their development than the females.'

The following note deals only with the order of hatching, Young (1922) having already dealt with the proportion of males to females. As it appeared possible that the food supply might influence the sexes differently, an attempt was made to breed the larvae on different food supplies, other factors being kept as nearly as possible equal. To do this, mixed batches of eggs were sunk and the larvae within twelve hours of hatching transferred to jars containing one of the following two food supplies:— (1) Minimum food supply, viz., tap water to which was added 0.015 per cent. polished rice and 0.5 gm. well-washed duck-weed to each 300 c.c.'s. water. (2) Maximum food supply, viz., stagnant river water filtered through fine wire gauze, to which was added 0.018 per cent. Peptone (Fairchild) and 0.5 gm. well-washed duck-weed to every 300 c.c. of water. Each larva was allowed 30 c.c. of the prepared water, this amount being regulated every day; thus to start with, ten larvae were placed in 300 c.c. of the food supply; if two died within twenty-four hours then the amount of water was reduced to 240 c.c. and so on. The results are shown in the following tables:—

TABLE III.

Maximum food supply. Number of larvae = 270.

Day of emergence ...	5	6	7	8	9	10	11	12	13	14	15	Total
Males ...	0	3	33	58	20	25	3	0	0	0	0	142
Females ...	0	1	10	35	24	24	3	1	0	0	0	98

TABLE IV.

Minimum food supply. Number of larvae = 305.

Day of emergence	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Males ...	0	5	17	19	9	10	11	6	9	9	5	2	0	0	0	0	1	1	0	1	0
Females ...	0	0	1	8	10	8	18	11	14	10	7	3	4	3	0	1	1	0	2	0	0

SUMMARY. In a mixed batch of ova, hatched and allowed to develop under food conditions which were either (1) favourable, or (2) adverse to growth, it was found that a much greater number of males than females reached maturity during the first few days of the emergences. This preponderance of males was greater than could be explained by the higher proportion of males to females (142 to 98, and 105 to 101) as observed in the completed experiments.

#### OVULATION

##### *Experiment I. Results of Diets other than Blood.*

Goeldi (1905), in Brazil, after numerous experiments with fruit, sugar, honey, etc., came to the conclusion that blood was necessary for the production of eggs by *Stegomyia calopus*. Fielding (1919), working in Australia with the same species of mosquito, obtained fertile eggs on three occasions on which peptone and sugar was given as a food. Ken (1917), in India, fed *Stegomyia scutellaris* on sugar, milk and sugar, peptone and sugar, with positive results.

*S. calopus* females were kept under observation for a period of over twelve months, at least twenty being always present in the cages. During this time the ordinary food supplied was sugar and water, and on two occasions a mixture of sugar and peptone was given for several days; the results were similar to those already published by Young (1922), no instance of egg laying being recorded.

Several authors record mosquitoes feeding on plants; thus Theobald (1901), states: 'I have frequently seen Culicidae settled on Compositae sucking the juices of the flowers, both males and females,' and Giles (1902), states: 'When mosquitoes are unable or unwilling to obtain blood they suck the juices of plants.' Knab (1907), quotes other instances. The following two experiments were made to see whether *Stegomyia* would feed on flowering plants, and if so, whether ova would result.

(1) Thirteen females were confined for thirty-eight days in a cage and supplied with water and a variety of flowering plants, representing as nearly as possible all the species growing within a ten-yards' radius of a heavily infected breeding place (a disused water barrel); in all seventeen species of plants were used, and each plant was allowed to remain in the cage for three to four days. In addition to the plants the fruits 'Goiaba' and banana were supplied. Males were always kept present. Both males and females constantly alighted on the flowers, inserted their proboscis in the corolla, and apparently absorbed some fluid.



(2) Seven females were observed under the same conditions for twenty-one days, but the following additional fruits were used : Melon, 'Mammão,' Mango, Orange, 'Periba,' 'Caju,' the results being the same as in the first experiment.

A trial was then made of the following native fruits : (3) Three females fed for thirteen days on Mango, 'Mammão,' Melon, Orange, Banana. (4) Five females fed for thirteen days on Mango, Orange, Banana, 'Periba,' 'Mammão.' No eggs were laid in either of the latter experiments.

SUMMARY. Female *Stegomyia* were offered and fed readily on sugar, sugar and peptone, flowering plants, and various fruits. No eggs were laid after feeding on any of the above substances.

*Experiment II. Results of feeding on animals and birds with special reference to Bats and Wall Geckos.*

Durham (1902), MacGregor (1915), Bacot (1916), Theobald (1916), and Fielding (1919), record *Stegomyia* feeding on Dog, Goat, Rat, Bandicoot, Agouti, and Guinea Pig; the results of the author's experiments of feeding *S. calopus* on various animals and birds are recorded in the following table.

TABLE V.

No.	Animal or bird used and method of feeding adopted.	Whether seen to feed	Ova laid	Whether fertile
1	A small finch confined in mosquito cage day and night on eight occasions ... ..	No	o	—
2	A rock-dove confined in mosquito cage day and night on four occasions ... ..	No	o	—
3	Young parrots; the tube containing the mosquitoes was applied to the host's body ... ..	Yes	+	+
4	Domestic chickens; method of feeding as above ... ..	Yes	+	+
5	A young otter; method of feeding as above. (Only a little blood absorbed, partly due to restlessness of animal) ...	Yes	o	—
6	Lesser Ant Bear; method of feeding as above. (Only half-hearted attempts made to pierce the skin) ... ..	No	o	—
7	Monkey; method of feeding as above ... ..	Yes	+	+
8	Cotia; method of feeding as above ... ..	Yes	+	+
9	Young Iguana ( <i>Urocentron azureum</i> ) confined in mosquito cage for some days and nights ... ..	No	o	—
10	Young Wall Gecko, placed loose in cage and also enclosed in tight fitting gauze bag; several trials day and night ...	No	o	—
11	Bats ( <i>Molossus obscurus</i> ) left loose in cage; several experiments tried both day and night ... ..	Yes	+	+

The above list requires no comment, except for the last two animals named. In all houses observed in Amazonas, whether deserted or occupied, two animals were constantly found present, viz., the gecko and various species of bats. Special attention was, therefore, devoted to seeing if *Stegomyia* would feed on these in the absence of human blood. The experiments with the gecko were frequently repeated, using both young and adult specimens. At first it was allowed loose in a cage of hungry mosquitoes, none of which attempted to bite. The gecko destroyed numbers of the mosquitoes, so in subsequent observations it was enclosed in a tight-fitting gauze bag and placed on the bottom of the cage. Though mosquitoes were often seen to alight on the gauze and probe it tentatively, they were never seen to draw blood, nor were any females gorged in the morning if the gecko and bag were allowed to remain in the cage over night.

The only record noted of mosquitoes feeding on bats is that of Durham (1902) at Pará, who observed a *Stegomyia calopus* female feed on 'a small bat (*Phyllostoma*).' The following species of bats were found to be common in or around houses in Manáos: *Saccopteryx bilineata*, Tenum., *Hemiderma perspicillatum*, L., *Vampyrops zarhinus*, H. All., *Molossus rufus*, Geoff., *Uroderma bilobatum*, Pet., *Molossus obscurus*, Geoff. Of these *Molossus obscurus* appeared to be the commonest in houses, and was used in the following three feeding tests. (1) Eleven offered a feed and nine fed; (2) three offered, two fed; (3) six offered, six fed. Not only did a far higher percentage of those given the opportunity feed on bats than on other animals, but they appeared to attack their host with a far greater voracity than they were observed to exhibit towards any other creature except man. They usually settled on and pierced the wing membranes, and as soon as they were flicked away returned to the attack, the complete feed being thus performed in a series of interrupted bites.

CONCLUSION. *Stegomyia calopus* feeds with great readiness and voracity on bats; it appears likely that these serve as important food reservoirs in deserted houses, or sparsely inhabited districts. It will also feed, but with less eagerness, on certain other animals and birds. All attempts to induce *Stegomyia* to bite the common wall gecko failed.

*Experiment III. Results of feeding on washed red cells, serum, and citrated blood.*

Otto and Neumann (1905), in Brazil obtained fertile eggs by feeding *Stegomyia* on blood and salt solution. Bacot (1916), in West Africa, on

two occasions obtained single eggs by feeding the mosquitoes, on one occasion on honey and blood, and on the other on syrup and blood, one of these eggs proving fertile. Marchoux and Simond (1906), at Rio imprisoned eight female *Stegomyia* and fed them as follows: Two on fresh human

TABLE VI.

No.	No. of female <i>Stegomyiae</i>	No. of days observed	Food: and how offered	No. of Ova laid
1	6	15	Washed sheep's cells in normal saline. 0.5 c.c. in a watch-glass ... ..	0
2	5	17	Washed sheep's cells in normal saline. 0.5 c.c. in a watch-glass ... ..	0
3	7	19	Sheep's serum. 0.5 c.c. in a watch-glass ... ..	0
4	7	24	Washed human red cells in normal saline. 0.5 c.c. in a watch-glass ... ..	0
4a	3	9	Washed human red cells in normal saline in tubes; same mosquitoes as in (4) ... ..	0
5	6	26	Human serum. 0.5 c.c. in a watch-glass ... ..	0
5a	3	5	Human serum, in tubes; same mosquitoes as in (5) ...	0
6	6	29	Whole human blood in saline. 0.5 c.c. in a watch-glass ...	0
6a	3	8	Whole human blood in tubes with normal saline; same mosquitoes as in (6) ... ..	0
7	3	10	Cotton ball soaked in whole human blood plus normal saline, suspended in cage ... ..	0
8	3	16	Cotton ball soaked in washed human red cells in normal saline, suspended in cage ... ..	0
9	3	13	Cotton ball soaked in human serum suspended in cage ...	0
10	2	14	Cotton ball soaked in washed human red cells plus normal saline, suspended in cage ... ..	0
11	3	8	Cotton ball soaked in whole human blood plus normal saline, suspended in cage ... ..	30*
12	3	12	Cotton ball soaked in human serum, suspended in cage ...	0

\* 29 hatched

serum, two on red cells separated by centrifuging, two on blood-clot, and two, which were used as a control, on themselves. The last two laid eggs, the remainder did not.

In the following experiment males were always present in the cages, and water supplied for the reception of eggs. The food was renewed every two or three days, in some instances every day. In Table VI (p. 434), under the column 'Food,' the expression 'in tubes' refers to the method used by Rodhain and others (1912) for feeding tsetse flies: small segments of glass tubing were covered at one end with the thin skin of a bat, filled with the food to be tested and suspended in the cage. This proved to be a very satisfactory method of feeding; the proboscis of the mosquito could be clearly seen piercing through the membrane into the fluid and sometimes the absorption of food could be observed; but neither by this nor by any other method, were the mosquitoes induced to feed to repletion as they do on the living animal.

SUMMARY. In a series of experiments in which fifty-four females were offered as food either serum, washed red cells, or whole blood (the two latter being diluted with normal saline), it was found that the mosquitoes absorbed any of the fluids offered, but that oviposition only resulted in the case of whole blood.

#### BITING OF CADAVERS

Rosenau and others (1904) quote two instances of *Stegomyia* feeding on native corpses, respectively half an hour and twelve hours after death. Christy (1900) observed mosquitoes (Anophelines) feeding on a white man's corpse in Nigeria three and a half hours after death.

The mosquitoes mentioned in the following notes were first tried on the living subject, and, if found willing to feed, were given the opportunity of biting the cadaver. All experiments were conducted in day-light.

(1) Native Brazilian, dead three hours. Three females used; all three bit, only one filled with blood.

(2) Native Brazilian, dead six and a half hours. Three females used; two bit, none absorbed blood.

(3) Native Brazilian, dead seven hours. One female used, which bit and absorbed blood.

(4) Native Brazilian, dead eighteen hours. Three females used; all three bit, none absorbed blood.

CONCLUSIONS. *Stegomyia calopus* females will bite corpses as long as eighteen hours after death, and will draw blood as long as six hours after death.



NATURAL ENEMIES OF *STEGOMYIA CALOPUS*

Young (1921) has dealt with the natural enemies of the larval *Stegomyia* and the value of dragon-flies in the destruction of the adults in Amazonas. The following note deals with the enemies encountered in dwelling-houses. Bacot (1916) considers toads, lizards, spiders, ants, scorpions, and possibly young Mantidae to be destroyers of *Stegomyia*. Marchoux and Simond (1906) at Rio found a jumping spider of the genus *Salticus* was a foe of *Stegomyia*, and came to the conclusion that it was probably of some practical importance. Neither of these authors publish any exact data.

In the experiments that follow an attempt was made to estimate the value of the animal as a mosquito destroyer, first by allowing it to feed on mosquitoes only, and then under conditions which gave it a choice between mosquitoes and other food likely to occur in its natural environment. Bats, wall geckos, and a species of jumping spider, were found to be common in all houses in Manáos. With regard to bats, twelve were killed in houses in Manáos; none of these showed any mosquito remains in the gut, nor were any scales found in the faeces.

*Experiment I.* A small wall gecko, about four and a half inches long was confined in a mosquito cage; the intervals between the feeds varied from a few minutes to several days.

TABLE VII

No.	<i>S. calopus</i>				<i>C. fatigans</i>				Other insects, Flies, Grasshoppers, etc.			
	At Start	After 4 hours	After 12 hours	After 24 hours	At Start	After 4 hours	After 12 hours	After 24 hours	At Start	After 4 hours	After 12 hours	After 24 hours
1	25	6	1	1	0	—	—	—	0	—	—	—
2	7	—	—	5	1	—	—	0	29	—	—	several
3	6	—	—	1	5	—	—	1	9	—	—	0
4	2	—	—	1	1	—	—	0	15	—	—	7
5	8	—	—	3	2	—	—	0	14	—	—	3
6	2	—	1	—	2	—	2	—	16	—	7	—
7	3	—	—	0	4	—	—	0	22	—	—	13



*Experiment II.* A jumping spider (Family *Salticidae*, genus *Akela*) which was found to be very common in all houses in Manáos, was confined either in a mosquito cage or in a small glass aquarium. As before, the intervals between feeds varied greatly.

TABLE VIII

No.	<i>S. calopus</i>				<i>C. fatigans</i>				Other Insects, Flies, Grasshoppers, etc.			
	At Start	After 4 hours	After 12 hours	After 24 hours	At Start	After 4 hours	After 12 hours	After 24 hours	At Start	After 4 hours	After 12 hours	After 24 hours
1	3	○	—	—	○	—	—	—	○	—	—	—
2	16	—	4	○	○	—	—	—	○	—	—	—
3	5	○	—	—	○	—	—	—	○	—	—	—
4	15	○	—	—	○	—	—	—	○	—	—	—
5	11	3	—	○	○	—	—	—	○	—	—	—
6	17	1	○	—	3	—	○	—	○	—	—	—
7	8	3	—	○	○	—	—	—	5	2	—	○
8	○	—	—	—	10	2	—	—	○	—	—	—
9	○	—	—	—	45	—	—	21	6	—	—	3

CONCLUSIONS. Both the wall gecko and a species of jumping spider (genus *Akela*), were found readily to destroy *S. calopus* and *C. fatigans*, and owing to their wide distribution are probably of some importance in limiting the numbers of mosquitoes occurring in human habitations. Their usefulness is, however, limited by the fact that other insects besides mosquitoes form part of their diet. An examination of twelve bats revealed no mosquito remains in the intestines.

**STEGOMYIA CALOPUS BREEDING NATURALLY ON BOARD A SHIP  
VOYAGING FROM MANÁOS TO LIVERPOOL**

Marchoux and Simond (1906) record placing 20 male and 20 female *S. calopus* in breeding tubes when leaving Rio in February, and on arrival at France, in May, twelve females and nine males were still alive. The insects during the voyage were fed on human blood.

The following observations were made in the month of February. Two days after leaving Pará a considerable number of *Stegomyia* were still to be found in various parts of the ship, and as their numbers appeared to be increasing, the whole ship was searched for possible breeding places. Eventually several hundred larvae were found in each of two large glass vases in the passengers' smoke room, both the jars containing cut-palm leaves which had been taken on board at Manáos. The larvae when found were at least forty-eight hours old, and as the steward stated that the vases had been cleaned and refilled about seven days previously, the ova must have been deposited shortly after leaving Manáos. When Havre was reached, sixteen days after leaving Pará, one jar contained many living imagoes, pupae, and larvae, the other, imagoes only. Liverpool was reached twenty days after leaving Pará, and twenty-four hours after the vessel had reached port, numerous living *Stegomyia* adults, both males and females, were present in both jars; in addition one of the jars contained a number of active pupae. The morning (2.0 a.m.) deck temperature varied between 81° F. at Pará and 53° F. in Liverpool.

CONCLUSION. *Stegomyia calopus* is capable of completing its cycle on board a ship travelling between Manáos and Liverpool, and the resultant adults can remain alive until arrival in Liverpool.

**NATIVE MOSQUITO REPELLENTS**

The natives who inhabit the forests surrounding Manáos are reported to employ two vegetable substances, 'Tocum' and 'Urucu,' to repel the attacks of biting insects. No opportunity occurred of testing the value of 'Tocum.' In regard to 'Urucu,' which Da Matta (1912) states is *Bixa orellana*, the seeds from the shrub of this name are crushed and added to nut oil, the resultant mixture being smeared over the exposed parts of the body. Da Matta (1912) agrees with the native belief that insects will not bite persons so protected. A sample was obtained and rubbed over

one arm, both arms being then thrust into a large mosquito cage, containing hungry *Stegomyia* females. The number of subsequent bites on each arm were as follows :—

Number of bites on arm treated with 'Urucu' ...	...	13
Number of bites on untreated arm ...	...	15

CONCLUSION. The native preparation known as 'Urucu' is of no value as a repellent of *S. calopus*.

#### ACKNOWLEDGMENTS

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## OBSERVATIONS ON THE RÔLE OF COCKROACHES IN DISEASE

BY

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As the cause of disease and as possible carriers of pathogenic organisms cockroaches have received less attention than other domestic pests, and less than might have been expected considering their wide distribution, their abundance, especially in tropical countries, their filthy habits and the opportunities they have of contaminating food, and the almost universal loathing with which they are regarded.

It is generally admitted that they are eminently fitted to be disseminators of infections, and from time to time they have come under suspicion as such, but hitherto no definite charge appears to have been brought home against them. There is, however, some experimental evidence that certain pathogenic organisms may be transported by them or may pass through their intestines. Morrell (1911), for example, as the result of his experiments, concluded that the insects are able by contamination with their faeces to bring about the souring of milk, to infect food and milk with intestinal bacilli, to transmit the tubercle bacillus, to disseminate pathogenic staphylococci, and to transmit from place to place destructive moulds. Longfellow (1913) showed that they may carry on their legs *Bacillus coli communis*, *B. proteus vulgaris*, *Staphylococcus aureus*, *S. citreus*, and a bacillus of the *subtilis* type, and found the same organisms in their faeces. He, therefore, considered that, as possible carriers of infection, it is almost as important to prevent the multiplication of cockroaches as of house-flies. This view, however, may be somewhat exaggerated, since Herms (1915) has pointed out that the feet of cockroaches are less well provided with spines and hairs than those of the house-fly, and are, therefore, less well adapted to the collection of filth. Barber (1912), working in Manila, has shown that cockroaches may be infected with plague bacilli, and more



recently (1914), that when fed on cultures or samples of human faeces containing cholera vibrios these organisms multiply in their intestines and are discharged in the faeces without losing their virulence. The cockroaches (*P. americana*) themselves are apparently unaffected, and Barber concludes that they may act as carriers of cholera to human food. According to Fibiger (1913), cockroaches (*P. orientalis* and *P. americana*) are also the intermediate hosts of a nematode (*Filaria* sp.) which causes malignant tumours in rats which feed upon them; and Wellman (1910) has suggested that a tapeworm (*Davainea* sp.) may be disseminated by these insects. As supplementing these examples, which are by no means complete, the following notes on a few experiments carried out at Accra may be of interest.

The species of cockroach employed in the experiments was *Periplaneta americana*, L. The insects were kept singly, in wide-mouthed glass jars, which were changed daily, and were fed on moist bread. Before submitting them to any experimental test, their faeces were examined carefully for several consecutive days in order to determine what natural infections they harboured. In the majority of the experiments, the material (faeces, etc.) which it was desired a cockroach should eat was offered to it smeared on bread. As soon as it had been consumed, the cockroach was transferred to a clean, dry jar and fed on moist bread, each pellet of faeces passed subsequently being examined during the following week, or longer period. In some experiments with bacteria, however, when it was necessary to prevent contamination of the limbs, etc., the cockroach was immobilised in a groove in a piece of cork (somewhat resembling a setting-board for lepidoptera) fixed by a layer of paraffin to a glass plate. In this manner the cockroach could be fed at the one end, and its faeces collected at the other without risk of contamination of the faecal pellets by the material used for feeding. The addition of a little carmine to the infecting feed was sometimes found to be of assistance as an indicator showing when the material had passed through the intestine.

The cockroaches usually passed one or two faecal pellets each day, which were either solid or semi-solid. Diarrhoea and the passage of liquid faeces was, however, by no means rare, and the insects were also liable to become constipated under the

conditions of the experiments. When forming their egg-capsules they frequently passed no faeces for several days in succession.

The faeces of the cockroaches always, or almost always contained innumerable bacteria of various types, yeast cells, moulds, fungal hyphae, &c., and in recently captured insects often also a considerable quantity of grit. After being in captivity for some days the number of yeast and fungal cells usually increased, and the bacteria became more numerous. A number of other parasites or coprozoic organisms were noted during the experiments. Perhaps the most common was a ciliate resembling *Balantidium blattarum*, Ghosh, the cysts of which were present in the majority of the cockroaches examined. *Oxyuris blattae* was frequently present, and in a few individuals were found *Entamoeba blattarum*, *Gregarina blattarum*, and a species of spirochaete. Mites,\* sometimes still alive, were found in the faeces on a few occasions. Thirty cockroaches were especially examined immediately after capture for eggs of worms known to frequent the intestine of man: in one a single egg indistinguishable from that of *Trichuris trichiura* was found. It should be stated, however, that most of the cockroaches were collected in the laboratory, where they would have little or no opportunity of feeding on human excrement.

#### TUBERCLE BACILLUS

Morrell (1911) has demonstrated that tubercle bacilli may be found in the faeces of cockroaches which have fed on infected sputum. In confirmation of this observation, three experiments were carried out in collaboration with Dr. A. Ingram and Dr. J. F. Corson.

The cockroaches, whose faeces had been previously examined and found to be free from acid-fast bacilli, were given sputum containing numerous tubercle bacilli from a case of phthisis in an African. They consumed the sputum very readily. Every sample of faeces passed subsequently, some of them fluid or semi-solid, was examined for *B. tuberculosis*, with the following results. The faeces passed on the first day after the infecting feed were free from the bacilli, those passed on the second day to the fourth or fifth day

\* These mites have been examined by Mr. S. Hirst, of the British Museum, who has kindly informed me that they are larvae of a Tarsonemid.

contained tubercle bacilli, and after this, up to the fourteenth day, when examinations were stopped, no more tubercle bacilli were detected.

The tubercle bacilli found in the faeces of the cockroaches stained in a normal manner and looked healthy. That they were actually living and virulent was proved by emulsifying one faecal pellet with normal saline solution and inoculating it into the groin of a guinea-pig, which in due course became infected with tuberculosis.

Cockroaches then feed readily on human sputum, and if the sputum contains tubercle bacilli, pass these organisms in their faeces for several days in a living and virulent condition. They do not appear to become infected with the bacilli themselves.

#### LEPROSY BACILLUS

In two similar experiments, cockroaches were given scrapings from the nose of a leper which contained numerous *B. leprae*. It was found that these bacilli also passed through the intestine of the insects, and appeared in the faeces for a day or two after the infecting feed. So far as could be judged from the appearance of the bacilli and from their staining properties, they had not been injuriously affected.

#### TYPHOID AND DYSENTERY BACILLI

It has been suggested by Scott (1915) that cockroaches may have acted as mechanical carriers of the infection in an outbreak of typhoid fever in Jamaica, and on other occasions elsewhere these insects have come under suspicion during epidemics of this disease and of bacillary dysentery. Cockroaches might also spread such infections by intestinal contamination. In order to determine if such bacilli, still living, could pass through their intestine, two experiments each were carried out with *Bacillus typhosus*, *B. paratyphosus*, B., and *B. dysenteriae* (Flexner Y).

The cockroaches used in the experiments had been previously tested carefully to see if their faeces contained any organisms resembling bacilli of the typhoid-dysentery group and had been found to be free from such infections. They were immobilised in a groove in a piece of cork with only their heads and tails projecting,

and were fed with small pieces of bread soaked with recent cultures of the bacilli to be tested. Their faeces were tested (in the routine manner) for the bacilli during the following week or ten days, but in none of the six experiments were they recovered.

These experiments, so far as they go, do not support the view that *B. typhosus*, *B. para-typhosus*, *B.*, and *B. dysenteriae* (Flexner Y) can pass unscathed through the intestine of the cockroach, but their number was too small to be conclusive. The faeces of the cockroaches contained a dense and varied bacterial and fungal flora, which may very well have out-grown the more delicate bacilli.

#### GONORRHOEA

In one experiment Gonococci were fed to a cockroach, and were subsequently sought for in its faeces. None were found either in direct smears or in cultures.

#### ENTAMOEBA HISTOLYTICA AND *E. COLI*

The cockroaches used in these experiments had previously been carefully examined for amoebic infections, a precaution which was doubly necessary, because some of these insects at Accra had been found naturally infected.

In four experiments cockroaches were given blood and mucous containing numerous actively motile *E. histolytica* from the stools of African patients suffering from acute dysentery. They consumed the samples readily, but neither amoebae nor their cysts were found in their faeces during the following week.

In nine experiments each human faeces containing cysts of *E. histolytica* and *E. coli* were fed to cockroaches. In seven of the former experiments cysts of *E. histolytica* were found in the faeces, and in seven of the latter experiments cysts of *E. coli*. The cysts were observed in the faeces usually for only one to three days, and eventually disappeared completely: they appeared to be healthy and unharmed by their passage through the cockroaches. No amoebae were found.

It would seem, therefore, that cysts of *E. histolytica* and *E. coli* can pass through the intestine of cockroaches without injury, and may thus be disseminated by these insects, but that they do not produce an actual infection in these hosts.



### ENTAMOEBA OF A MONKEY

In another experiment entamoebae resembling *E. coli* from the faeces of a monkey (*Cercopithecus patas patas*) were given, but no entamoebae or cysts were subsequently found in the faeces of the cockroach. A few days later the faeces of the same monkey, which now showed numerous eight-nucleated and a few sixteen-nucleated cysts, were fed again to the same cockroach. On the following day no faeces were passed by this cockroach, but on the second and third days its faeces contained numerous cysts, similar to those in the monkey's faeces, which appeared to be healthy and were not stained by eosin. On the fourth day the cysts were fewer, and on subsequent days none were found.

### GIARDIA

In two experiments cysts of *Giardia intestinalis* fed to cockroaches in human faeces passed through their intestines apparently unharmed and unchanged.

### EGGS OF WORMS

A number of experiments were carried out to determine what was the effect on the eggs of worms of passage through the intestine of cockroaches. The eggs were given in human faeces smeared on bread. The results of the experiments may be summarised as follows.

*Hook-worms.* Seven experiments. The eggs of *Ancylostoma duodenale* and *Necator americanus* passed through the intestine unharmed, and appeared in the faeces for from one to three days after the infecting feed, the time depending on the rate of passage of the intestinal contents, which was variable. They had undergone some development, many of those found in the cockroaches' faeces containing living embryos, which subsequently hatched when the faeces, with a little saline solution, were mixed with charcoal and kept at the laboratory temperature. It may be added here that other experiments showed that eggs of *Ancylostoma ceylanicum* in dogs' faeces fared similarly when fed to cockroaches.

*Ascaris.* Five experiments. The eggs of *Ascaris lumbricoides* passed through the intestine and appeared in the faeces of the cockroaches for a day or two. They appeared to be unharmed. In most cases they also were unchanged, but in one experiment, in



which they did not appear in the faeces until the fourth day owing to the fact that the cockroach was constipated, they had undergone slight development, their contents having divided.

*Trichuris.* Eight experiments. The eggs of *Trichuris trichiura* passed through the intestine with the residue of the infecting feed, and appeared in the faeces apparently unharmed and usually unchanged, but sometimes having undergone slight development, their contents having divided. Eggs were occasionally found also a day or two later, and in this case they were usually empty shells. For example, a cockroach fed with human faeces containing *Trichuris* eggs showed in its faeces on the following day numerous healthy-looking, unsegmented eggs, on the next day a single, healthy-looking, unsegmented egg, on the third and fourth days no eggs, but on the fifth day two empty shells.

*Taenia.* Four experiments. The eggs of *Taenia saginata* used appeared in the faeces of the cockroaches apparently unchanged in two of the experiments, in one only shrunken eggs were observed, and in the fourth none were found. The eggs were scanty in the sample of human faeces used in these experiments.

*Bilharzia.* No opportunity has occurred of experimenting with the eggs of *Schistosoma mansoni*. In one experiment, however, urinary deposit containing numerous eggs of *S. haematobium* was fed to a cockroach on bread. The eggs, apparently unchanged, appeared in the faeces of the insect on the following day.

From these experiments it is clear that the eggs of many intestinal worms may pass unharmed through cockroaches, and as these insects readily feed on human faeces they may aid in the dissemination of these parasites. In some cases the initial stages of development took place in the eggs during their sojourn in the cockroaches.

#### **APHIOCHAETA XANTHINA**

The larvae of the small fly *Aphiochaeta xanthina*, Speiser, which belongs to the Family *Phoridae*, are known to cause intestinal myiasis in man in the Gold Coast, it being supposed (Patton, 1922) that 'its eggs and larvae probably gain entrance to the human alimentary tract in food, and particularly in stale meat.' In a single experiment eggs of this fly were given to a cockroach on moist

bread, and were eaten by it. On the second and third days following the experimental feeding the faecal pellets passed by the cockroach were found to contain fragments of the eggs and one or two almost complete eggs which appeared to be empty. Thereafter, up to the fourteenth day, when examinations were discontinued, no evidence was found of the presence of the fly in any stage of its development.

### SUMMARY

The following organisms appeared to pass unharmed through the intestine of the cockroach *Periplaneta americana*:—*Bacillus tuberculosis*, *B. leprae*, cysts of *Entamoeba histolytica*, *E. coli* and of an entamoeba of a monkey resembling *E. coli*, cysts of *Giardia intestinalis*, and eggs of *Ancylostoma duodenale*, *A. ceylanicum*, *Necator americanus*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Taenia saginata*, and *Schistosoma haematobium*.

On the other hand Gonococci, *Entamoeba histolytica*, *E. coli*, and an entamoeba of a monkey resembling *E. coli* (in the vegetative stages), eggs of *Aphiochaeta xanthina*, and, in two experiments each, *Bacillus typhosus*, *B. para-typhosus*, B., and *B. dysenteriae* (Flexner Y) were not recovered in the faeces of cockroaches after experimental feeding.

No evidence was obtained that any of the organisms used in the experiments established themselves as parasites in the intestine of the cockroaches.

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## THE OCCURRENCE OF *XENOPSYLLA* *ASTIA*, ROTHs., IN WEST AFRICA

BY

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Collections of rat fleas made at Accra, Gold Coast, during the months August, 1921, to July, 1922, inclusive, have been received for identification from Dr. A. Ingram.

Among the April and May samples were specimens of *Xenopsylla astia*, Roth. This species has not, as far as I am aware, been found hitherto in Africa: it is, therefore, thought desirable to place its occurrence at Accra on record.

The data are as follows:—

From *Mus decumanus*, April, 1922, *X. astia*, ♂♂ 9; ♀♀ 13.

From *Cricetomys gambianus*, April, 1922, *X. astia* ♀ 1;

May, 1922, ♂ 1; ♀ 1.



## NOTES ON A CASE OF BLACKWATER FEVER

BY

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*(Received for publication 27 November, 1922)*

The writing of this note has been prompted by some remarks of Professor Warrington Yorke (1922) in a critical review of recent work on the pathology of blackwater fever. These will be referred to later.

### CLINICAL HISTORY

J. D. T., aged 28, suffered from no disease of importance until June, 1921, when he contracted malaria in West Africa. This was a slight attack. In February, 1922, he had a severe attack of malaria, with which he was invalided home. He arrived in England on March 20th, and remained comparatively well, taking a small dose of quinine daily, till April 10th. On that day he felt shivery, and went to bed after mid-day. In the evening he took 15 grains of quinine. Next morning (April 11th) he still felt out of sorts, and at 11 a.m. passed dark red urine. Similar urine was passed at 1 p.m. I saw him in the early evening of the same day, and he was admitted to Professor T. K. Monro's wards in the Western Infirmary of Glasgow a couple of hours later. He looked rather ill, with temperature  $102.6^{\circ}$ , headache, and some yellowness of the skin. The spleen was enlarged, and the blood-film showed a few subtertian malarial parasites. A condition of suppression of urine had apparently set in, for, though he had passed no urine for eight hours, there was no desire to micturate, and the bladder was not distended. A few cubic centimetres of blood were taken for examination, and then 1,800 c.c. of 1 per cent. saline was injected intravenously. This injection, together with three litres of fluid taken by the mouth during the night had the effect of re-establishing



the flow of urine, and within twelve hours he passed 2,200 c.c., coloured dark red. Throughout the rest of the illness the output of urine was good, the lowest recorded in any twenty-four hours being 1,100 c.c. (April 28th). Haemoglobinuria continued for four days, but by April 15th there was no reaction with guaiac. The patient, however, was becoming increasingly ill, and for two days (April 15th-17th) it looked as though he were going to die. He was delirious, anaemia was intense (Hb. 16 per cent. on April 16th), and there was remittent pyrexia which continued to range from  $99^{\circ}$  to  $103^{\circ}$  or  $104^{\circ}$  until April 18th. Thereafter the temperature did not exceed  $100^{\circ}$  except for twenty-four hours on April 21st-22nd, when it reached  $102.4^{\circ}$ . This coincided with a recurrence of haemoglobinuria for a similar period. It became normal on April 25th, and remained so during convalescence except for a rise on May 6th-8th from a relapse of malaria, during which a few subtertian rings were again found in the blood. He was dismissed hospital well on June 2nd.

#### *Urine*

The state of the urine is here shown:—

Date			Colour	Specific gravity	Guaiac test
April	11	...	dark red	1018	+
"	12	...	"	1015	+
"	13	...	dark amber	1020	+
"	14	...	amber	1020	—
"	15-20	...	"	...	—
"	21	...	dark amber	1022	+
"	22	...	dark red	1025	+
"	23 onwards		amber		—

The specimens of red urine had a copious brownish deposit, which showed brown casts under the microscope. No red corpuscles were seen. Spectroscopic examination showed the bands of oxy-haemoglobin.

#### *Blood*

The specimen of blood taken on admission to hospital was run into a dry tube and was allowed to clot. The serum which separated was dark red in colour. It was not matched with a standard, but was much darker than the tube of a Gowers'

haemoglobinometer. The spectrum was that of oxy-haemoglobin. The urea in this sample of serum was estimated, and was found to amount to 84 mgm. per 100 c.c. of blood. The method employed for the estimation was that described by Kennaway (1920), which depends on the power of an enzyme in the soya bean to break up urea quantitatively into ammonium carbonate, and briefly is as follows. The serum is treated with alcohol to remove the protein, and then is diluted with water. A few drops of a methyl red solution are added as an indicator, and the fluid is brought to a buff shade which corresponds to a constant acidity. A control flask containing water is brought to the same shade, that is, to the same reaction, and to each is added a watery extract of powdered soya bean. The flasks are incubated in a water-bath for an hour, and then by titration the difference in the acidities is ascertained. This difference is due to the production of ammonium carbonate in the serum, and the degree of alkalinity produced indicates the amount of urea originally present. Full details of the method will be found in the original paper. Normal blood gives a reading of 30 to 35 mgm. of urea per 100 c.c.

A few blood-counts were done:—

Date	Hb %	Red Cells	Leucocytes
April 16	... 16	1.3 m.	14,000
„ 26	... 30	1.9 m.	11,000
May 7	... 52	3.5 m.	...
„ 15	... 60	3.8 m.	...
„ 21	... 76	4.2 m.	...

*m = million.*

A film taken when the anaemia was severe (April 17th) showed marked anisocytosis of red cells, with some megalocytes. Nucleated reds numbered five hundred per c.mm. A differential leucocyte count showed neutrophil polymorphs 77 per cent., lymphocytes 18 per cent., large hyalines 2 per cent., eosinophil polymorphs 3 per cent. Immature polymorphs were numerous. By May 21st the blood-film was practically normal.

Examination of thick films for malarial parasites showed:—

On April 11	...	A few subtertian rings
„ 13	...	No parasites
„ 15	...	„ „
May 6	...	A few subtertian rings
„ 21	...	No parasites

*Quinine*

The following doses of quinine sulphate were given by the mouth:—

April 17	...	gr. 3
„ 18, 19	...	gr. 6 daily
May 6, 7	...	gr. 5 daily
„ 8 onwards		gr. 10 daily

**REMARKS**

A point of interest in this case is the occurrence of marked haemoglobinaemia on the day on which haemoglobinuria began. In the review referred to, in the first paragraph, Yorke writes: 'There is unfortunately astonishingly little in the way of precise information on this important point' [the presence of haemoglobinaemia], and he quotes from Christophers and Bentley: 'Examination of the blood in blackwater fever has shown without exception the presence of true haemoglobinaemia, demonstrated by the centrifuging of blood received into hypertonic citrate solution; the serum after clotting has also always shown haemoglobin . . . but in both cases the amount was usually small, and more or less masked by the extraordinarily intense yellow coloration of the serum.' In the case I have described the serum was dark red.

Yorke raises the question also as to whether Plehn is correct in stating that the urine in blackwater fever is of extraordinarily low specific gravity, and mentions numerous instances in favour of the opposite view. In the case of J. D. T., the four 24-hours' specimens of red urine showed a specific gravity of 1018, 1015, 1020, and 1025.

A further point of interest is the rise in the blood urea at the end of a quite short period of suppression of urine.

As regards treatment, it seems to me that in this case the injection of saline intravenously when failure of urinary secretion showed itself had much to do with the re-establishment of the flow. I have made this observation before (1918).

As has been pointed out to me by Professor C. H. Browning, there is a striking difference between the symptomatology of blackwater fever and that of paroxysmal haemoglobinuria. In the latter condition the patient may be disturbed hardly at all by an attack of haemoglobinuria, whereas in the former the disturbance is

profound. In the case I have described, it was noticeable that not only did the patient not improve with the cessation of haemoglobinuria, but he grew worse for two days, with continuation of the pyrexia, and for four days at least after the urine had become clear his condition was critical.

### SUMMARY

A case of blackwater fever is described in which haemoglobinaemia was marked. The blood urea was found to be much increased after a short period of anuria.

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## CASE OF TRYPANOSOMIASIS

BY

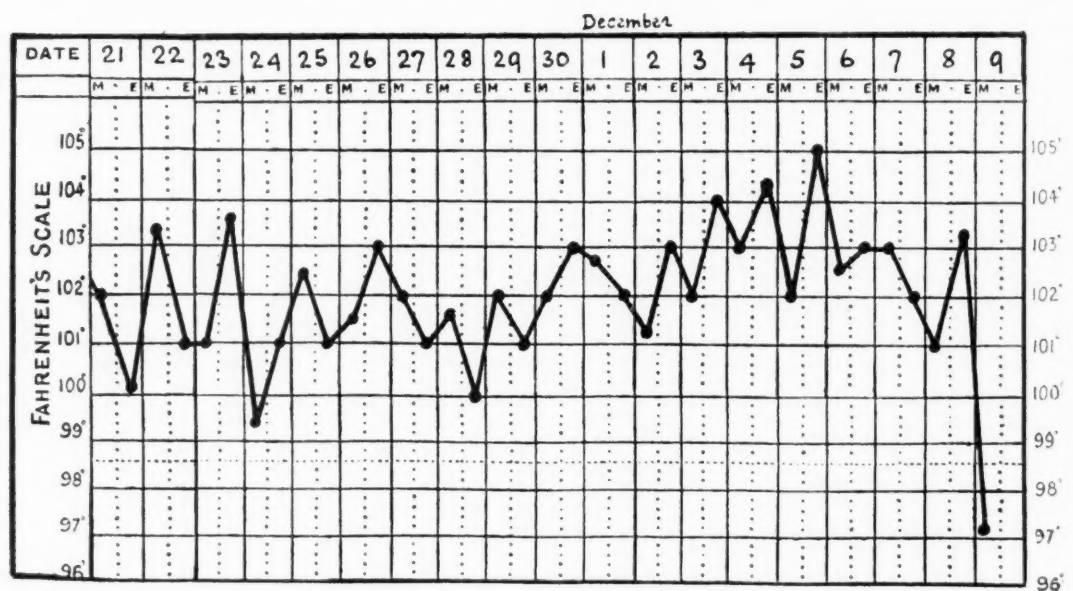
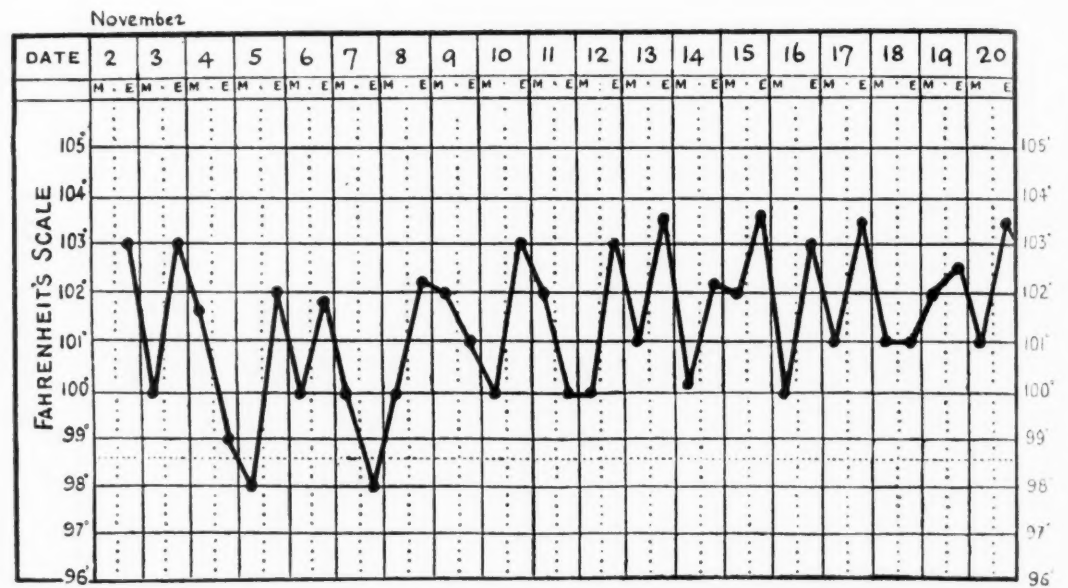
A. J. MACKENZIE, M.B., Ch.B. (Edin.)

RHODESIAN MEDICAL SERVICE

*(Received for publication 27 November, 1922)*

The following notes are published, as the case was one of interest.

The patient, a married European woman about 18 years of age, had, in August, 1919, accompanied her husband on a shooting expedition in a tsetse-fly area in Sebungwe, S. Rhodesia. After she had been on the veldt six or seven weeks she became ill, and was taken to the nearest town and treated for malaria for three weeks. As at the end of this time her condition had not improved, she decided to return to her own home. On admission to hospital on October 31st she was evidently acutely ill, and had a temperature of 102°. She complained of intense headache and severe pain all over the body. The spleen was slightly enlarged, and the posterior cervical glands were also slightly enlarged and tender on pressure. A blood smear taken on admission and stained with Giemsa showed a very severe trypanosome infection. The trypanosome appeared to be *T. brucei* vel *rhodesiense*. Posterior nuclear forms were found in the patient's blood. This diagnosis was confirmed by intraperitoneal inoculation of the patient's blood into a rabbit. Treatment with galyl, soamin and tartar emetic had no effect, and the patient gradually became worse. Hyperaesthesia was a marked feature of the case from the outset. The slightest touch made her cry out with pain. Her mentality changed, and she became childish and played with dolls. About the middle of November she developed keratitis, which affected both eyes. This became worse, and in a fortnight she was almost completely blind. Emaciation was progressive throughout the illness. She gradually sank, and became comatose two days before her death, which occurred on December 9th.



Case of Trypanosomiasis: Chart.

## OBSERVATIONS ON *ONCHOCERCA* *VOLVULUS*

BY

J. W. S. MACFIE

AND

J. F. CORSON

(Received for publication 28 November, 1922)

The following brief and somewhat disconnected notes on *Onchocerca volvulus* are based on observations made at Accra in the Gold Coast, West Africa.

DIAGNOSIS. The tumours of *O. volvulus* are by no means always large and easily recognisable, but are frequently very small, deep-seated, and difficult to detect. In some cases, indeed, we have been able to palpate them only after they had been located for us by the patients themselves. The diagnosis of volvulosis by the presence of tumours is, therefore, unreliable, and we have found it more satisfactory to examine the skin for larvae.

The method we adopt is to remove from the lower part of the back a small piece of skin similar to, but rather larger than, those used in skin-grafting by Reverdin's method. The skin is raised with the point of a needle, and a piece of the required size snipped off with a pair of sharp scissors. The pieces of skin may be examined immediately by teasing them on a slide with a little normal saline solution, or they may be left for an hour or two in saline solution in small tubes, in which case the larvae will be found to have worked their way out and to be lying at the bottom, or they may be used for sectioning. The technique is simple and rapid, and as it is not painful and is not objected to by African patients, is capable of wide application. The little wounds heal rapidly.

In such pieces of skin removed from patients with *O. volvulus* tumours we have invariably found larvae. In the one or two apparent exceptions met with, the tumours on removal proved to be juxta-articular nodules, and not *O. volvulus* tumours. In many other cases in which no tumours could be found, the pieces of skin removed in this way contained *O. volvulus* larvae.

As regards 'lichenification' and the other skin conditions

sometimes considered to be due to volvulosis, we have on the one hand observed them in skin in which no larvae were found, and on the other hand found larvae abundantly present in apparently normal skin. Moreover, we have recently found another filarial larva in the skin which, at Accra at any rate, is even more commonly present than that of *O. volvulus*. In view of this discovery, further observations are necessary before it can be said if either of the two larvae is responsible for the lesions.

INCIDENCE. In order to obtain some idea of the prevalence of *O. volvulus* infection in the Gold Coast, fifty men, taken at random, were examined at Accra, all of whom were adults, between the ages of 25 and 45 years, who appeared to be in good health. The examinations were made on the 24th of October, 1922, between the hours of 9.45 and 10.15 a.m.

From each man a small piece of skin, as described above, was removed from the small of the back and placed in a tube containing about 2 c.c. of normal saline solution. The piece of skin was subsequently teased up together with a drop or two of the saline solution from the bottom of the tube, the preparation fixed by heating, dried, and stained with haemalum. In no case was there obvious blood in the specimen.

The result of this examination was that larvae of *O. volvulus* were found in seventeen of the men (equal to 34 per cent.).

PERIODICITY. Ten of the men referred to above, in whom larvae had been found, were re-examined two days later at about 9 p.m. No sensible difference was observed suggestive of a periodicity in the prevalence of the larvae in the skin. This observation is in harmony with that of Montpellier and Lacroix (1920).

DISTRIBUTION OF THE LARVAE IN THE BODY. In most of the cases examined we have sought for the larvae of *O. volvulus* in the skin of the lumbar region or the small of the back only. In a few instances, however, we examined other parts also; for example, in a Kru man with a small tumour in the left inguinal region, larvae were found abundantly in the skin of the left buttock, the right ankle, the right shoulder cap, and the right wrist. Our observations, indeed, showed clearly that even in subjects in whom no tumours could be detected and whose skin was normal, larvae of *O. volvulus* might be found in the skin of widely separated regions of the body.

TABLE

The distribution of *O. volvulus* larvae in the body.

Parts of the body examined	I Kru man, c. 25	II Ashanti man, c. 40	III Ashanti man, c. 40
Skin of scalp : left occipital region ...	nil	—	—
above the right ear ...	—	—	nil
Skin, behind the left ear ... ..	—	mf. v. numerous	—
Skin : right wrist ... ..	—	—	nil
left wrist ... ..	mf. v. numerous	—	—
Skin, second finger of right hand ...	—	nil	—
Skin, small of back ... ..	mf. v. numerous	mf. v. few	mf. v. numerous
Skin, scrotum ... ..	—	—	mf. v. few
Skin : right ankle ... ..	mf. v. numerous	mf. v. numerous	—
left ankle ... ..	—	—	mf. v. numerous
Mucous membrane of mouth, lower lip..	nil	nil	nil
Stomach ... ..	nil	nil	—
Small intestine ... ..	nil	nil	—
Large intestine ... ..	nil	nil	—
Rectum ... ..	—	—	nil
Mesentery ... ..	nil	nil	nil
Parietal pleura, 8th interspace ... ..	nil	nil	—
Intercostal muscle, 8th interspace ...	nil	—	—
Lung ... ..	nil	nil	nil
Heart, left ventricle ... ..	nil	nil	—
Aorta ... ..	nil	nil	nil
Peritoneum... ..	nil	nil	—
Liver ... ..	nil	nil	nil
Spleen ... ..	nil	nil	nil
Kidney ... ..	nil	nil	—
Bladder ... ..	nil	nil	—
Brain : cerebral cortex ... ..	—	—	nil
cerebellum ... ..	—	—	nil
Lymphatic glands : near aorta ... ..	nil	—	nil
mesenteric ... ..	nil	nil	nil
inguinal ... ..	nil	—	nil

mf. v. = larvae of *Onchocerca volvulus*.

nil = no larvae found.

— = not examined.



In order to ascertain more accurately the distribution of the larvae in the body, three natives were examined particularly in the mortuary, the first a Kru man, aged about 25 years, and the second and third Ashanti men, aged about 40 years. The first two men had died from pulmonary tuberculosis, and the third from cerebral congestion. No tumours were found in any of the three, and no definite skin lesions of the types associated with volvulosis, excepting in the third man, who had slight 'lichenification' of the back.

The various parts of the body examined, and the results, are shown in the accompanying table. About 0.25 c.c. of each tissue was examined. It will be noted that larvae were found in the skin of widely separated areas, but that they were not found in any of the mucous membranes or organs.

During the autopsies a careful search for adult worms was made in the mesentery and the retro-peritoneal tissue in the neighbourhood of the liver, the duodenum, and the aorta, but none were found. The inner surface of the aorta (in view of the fact that *O. armillata* is abundant in this situation in cattle at Accra), and a number of lymphatic glands from the mesentery, the inguinal region, and near the aorta were also examined without success.

LARVAE ARE NOT FOUND IN SWEAT. Although the larvae of *O. volvulus* are abundant in the skin, they do not appear in the sweat. One of the laboratory staff, a Mendi, in whose skin larvae were numerous, was set to work in the sun until he perspired freely. Sweat was then collected from his face, chest, abdomen and back, and examined for larvae. None were found.

EXPERIMENTS WITH TSETSE-FLIES. Leiper (1913) failed to trace any development of the larvae of *O. volvulus* in *Stemoxys calcitrans* and *S. nigra*, and Rodhain and Van den Branden (1916) failed with *Stegomyia fasciata* and *Cimex rotundatus*. Brumpt has suggested that the larvae may develop in a tsetse-fly, but so far as we are aware, no observations have yet been recorded in support or otherwise of this view. A few experiments were, therefore, carried out at Accra, in which wild tsetse-flies were fed on patients in whose skin *O. volvulus* larvae were abundant, and subsequently dissected and examined for developmental stages of these parasites. Unfortunately for our purpose Accra is situated in an extensive

tsetse-free area, and we were, therefore, able to procure only a few living flies for our experiments.

*Glossina palpalis*, R. D. Three flies were fed once only on a case of volvulosis and dissected, two on the sixth day, and one on the seventh day after the infecting feed. No filarial larvae were found. Ten specimens which had not been fed experimentally were also dissected as a control. No filarial larvae were found in them.

*G. longipalpis*, Wied. Six flies were fed once only on a case of volvulosis and dissected, two on the twelfth day, and one each on the second, fourth, fifth and sixteenth days after the infecting feed. No filarial larvae were found. Fifteen specimens which had not been fed experimentally were also dissected as a control. No filarial larvae were found in them.

These few observations do not support Brumpt's suggestion, so far, at any rate, as concerns *G. palpalis* and *G. longipalpis*. The number of flies employed was, indeed, regrettably small, but if volvulosis is as prevalent as the figures we have given suggest, and if the parasites are able to develop in them, it might have been expected that one or two of these tsetse-flies (including the controls) might have shown them.

EXPERIMENTS WITH LICE. From the usual position of the larvae in the skin, namely, close under the rete mucosum, we are inclined to think that the intermediate host, if indeed it is a biting insect, will prove to be one which does not probe the skin deeply. Lice at once suggest themselves, but so far as our observations have at present proceeded we are not able to incriminate them. One of us (J. F. C.) dissected about sixty specimens of *Pediculus humanus corporis* at Sekondi without finding any filarial larvae, and further dissections (forty-six) at Accra have been equally fruitless. Moreover, twenty lice taken from the bodies of two men not infected with *O. volvulus* and fed on a man who harboured larvae of *O. volvulus* in his skin, and larvae of *Acanthocheilonema perstans* in his blood, were dissected an hour later. Larvae of *O. volvulus* were not found in any of them, but living and active larvae of *Ac. perstans* were observed in eight. This experiment suggests that the lice, in feeding, draw up the larvae present in the blood of their host, but not those in his skin. It may be added that in several of the lice dead and partly digested larvae of *Ac. perstans* were found

(derived, presumably, from some previous host), an observation which confirms that of Low (1903), who failed to trace development of this worm in *P. capitis* and *P. vestimentorum*.

From the fact that the larvae are particularly numerous in the skin at the base of the trunk (buttocks, scrotum, &c.), *Phthirius pubis* might be regarded as a likely host. Contrary to expectation, these creatures have proved difficult to obtain at Accra, and we have not yet been able to procure any for dissection and experiment.

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## A NEW SPECIES OF FILARIAL LARVA FOUND IN THE SKIN OF NATIVES IN THE GOLD COAST

BY

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AND

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(Received for publication 28 November, 1922)

When investigating the occurrence of larvae of *Onchocerca volvulus* in the skin of natives in the Gold Coast, sheathless larvae of another species of Filariidae were found in several cases. So far as we are able to ascertain these larvae have not previously been noted, and, therefore, notwithstanding the fact that we have not yet discovered the adults, a brief description of them is given here.

The larvae were found in the skin of nine out of twenty-four cases selected for examination for *O. volvulus* larvae either because they had tumours, or because the skin showed the conditions which have been associated with that infection. From each of these cases a piece of skin, about half a square centimeter, was removed from the small of the back and placed in a tube containing normal saline solution. The larvae, which were found in the deposit which collected at the bottom of the tubes, were fixed by adding Ruge's solution to some of the deposit on a slide, allowed to dry, and subsequently stained with haemalum. The larvae were also found in the skin of the forearm and back in one out of nine unselected autopsies. In this case the larvae were fixed on a slide by heating, dried, and stained with eosin-azur.

The description of the larva which follows is based on the examination of specimens from these ten cases. Seventy-two larvae were measured, ten from each case in which this number could be found, and all that were available in the others. It may be noted here that there was a certain degree of variation in the size of the larvae in different cases, in some they were on the average slightly larger than in others.

**MORPHOLOGY.** The larvae are sheathless, slender, tapering both anteriorly and posteriorly, and when fixed assume a characteristic form, the body being straight, or nearly so, excepting at the posterior extremity, which is curved round like the handle of a walking-stick (see fig. 1, A). The cuticle is striated. The nuclei are

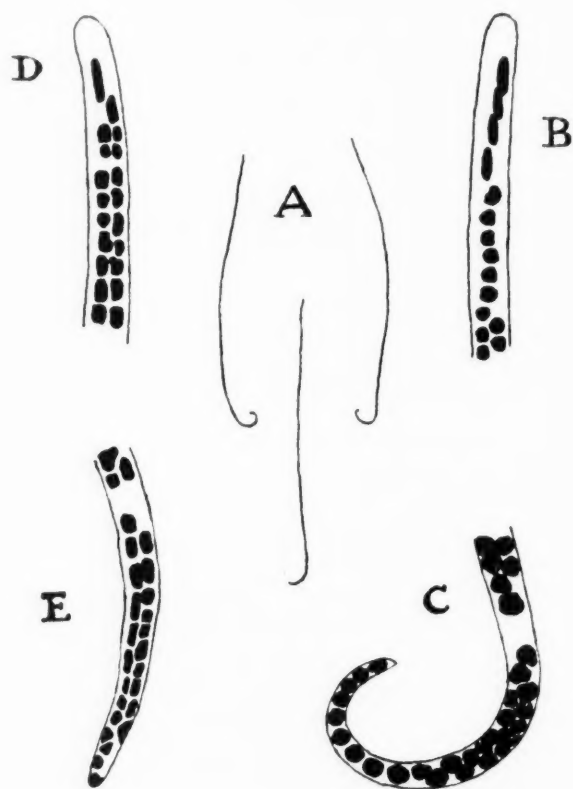


FIG. 1.—A. The larvae,  $\times$  c. 150, to show the general form; B and C. The anterior and posterior extremities  $\times$  c. 1375; D and E. The anterior and posterior extremities of the larva of *Ac. perstans*,  $\times$  c. 1375, for comparison with B and C.

rather large, two or three abreast in the middle of the larva, and completely fill the greater part of the body.

*Length.* The lengths of the seventy-two larvae measured ranged from  $180\mu$  to  $240\mu$ , average  $215.5\mu$ . The table shows that nearly 60 per cent. were between  $210\mu$  and  $229\mu$ .

*Breadth.* The breadth at the widest part of the body is about  $3\mu$ .

*Anterior extremity.* The body tapers very slightly towards the anterior extremity, and is bluntly rounded at its end. No 'fang' could be distinguished. The clear area at the anterior end is about  $4\mu$  long. The column of nuclei commences with a single row of ten



or twelve nuclei, the first four being usually oval and the others somewhat quadrate.

The distribution according to lengths, and to the position of the nerve ring, of seventy-two of the filarial larvae.

Lengths, in microns		Nerve Ring : distance from anterior extremity, in microns.	
180 $\mu$ to 189 $\mu$ ... ..	5	40 $\mu$ to 44 $\mu$ ... ..	—
190 $\mu$ to 199 $\mu$ ... ..	6	45 $\mu$ to 49 $\mu$ ... ..	2
200 $\mu$ to 209 $\mu$ ... ..	7	50 $\mu$ to 54 $\mu$ ... ..	17
210 $\mu$ to 219 $\mu$ ... ..	22	55 $\mu$ to 59 $\mu$ ... ..	28
220 $\mu$ to 229 $\mu$ ... ..	21	60 $\mu$ to 64 $\mu$ ... ..	22
230 $\mu$ to 239 $\mu$ ... ..	10	65 $\mu$ to 69 $\mu$ ... ..	2
240 $\mu$ to 249 $\mu$ ... ..	1	70 $\mu$ to 74 $\mu$ ... ..	1

*Other anatomical fixed points.* The nerve ring is situated about 26.9 per cent. of the length from the anterior extremity: it is a well marked break, in the middle of which is a single, prominent nucleus. In the seventy-two individuals measured, its position varied from 48 $\mu$  to 71 $\mu$ , average 58 $\mu$  from the anterior extremity. The excretory pore is small, and is situated about 34.1 per cent. of the length from the anterior extremity; the excretory cell lies slightly more posteriorly. The G1 cell, which is not always easily recognised, is large, with a round nucleus, and situated about 69.2 per cent. of the length from the anterior extremity. The anal pore is a small break in the column of nuclei situated about 86.2 per cent. of the length from the anterior extremity. A central viscus was not seen.

*Posterior extremity.* The body tapers for a considerable distance towards the posterior extremity, and the extreme tip of the tail, beyond the last nucleus, is abruptly pointed so that the posterior clear area is at most about 1 $\mu$  long. The tail is curved sharply into a crook, and the column of nuclei at its extremity is a single row of rounded, or at most oval, nuclei.

**SITES WHERE THE LARVAE WERE FOUND.** The larvae were found only in the skin. The part examined in nine of the cases was the

small of the back, and in one case the right forearm and the back between the blades of the scapulae. In the latter case skin from the abdomen near the umbilicus and from the middle of the outer side of the calf of the left leg were also examined, but no larvae were found in these situations. In nine cases six or more blood films from the finger and from the back (near to the spot where larvae were found in the skin) were examined; and in two of these four thick films taken at night, and in two others 3 c.c. of blood taken during the day, were also examined. In none of these specimens were the larvae found. Larvae of *Acanthocheilonema ferstans*, however, were found in two.

In one post-mortem examination in which the larvae were found in the skin of the small of the back, the following parts of the body were also examined, but without discovering any larvae: skin of scalp above the right ear, skin of right wrist, skin of left ankle, skin of scrotum, mucous membrane of the mouth, rectum, lung, aorta, liver, spleen, cerebral cortex of brain, cerebellum, and lymphatic glands along the aorta, in the mesentery, and in the right inguinal region.

Sections of the skin showed the larvae lying in the tissue spaces of the cutis vera or corium, usually close to the rete mucosum. There was present in all the cases examined a slight degree of cellular infiltration, especially round the blood vessels, but with this exception no definite departure from the normal condition was observed.

**PATHOGENICITY.** Our observations, which were made in the course of an investigation of volvulosis, do not admit of any statement being made as to the effects which may be caused by infection with this parasite. It may be noted, however, that the condition of the skin known as 'lichenification' was present in six of the ten cases examined, and a definite thickening in two others. Larvae of *O. volvulus* were present in the skin of five cases, but were absent from four of the six which showed 'lichenification.' It is, therefore, possible that the presence of the larvae in the skin may cause irritation and lead to pathological changes.

**INCIDENCE.** In order to gain some idea of the prevalence of this filarial infection in the Gold Coast, fifty men, taken at random, were examined at Accra. All the subjects were adults between the

ages of 25 and 45 years, who appeared to be in good health. The examinations were made between 9.45 and 10.15 a.m. on the 24th of October, 1922.

From each man a small piece of skin, similar to those taken for skin-grafting by Reverdin's method, was removed from the small of the back and placed in a tube containing about 2 c.c. of normal saline solution. The piece of skin was subsequently teased up

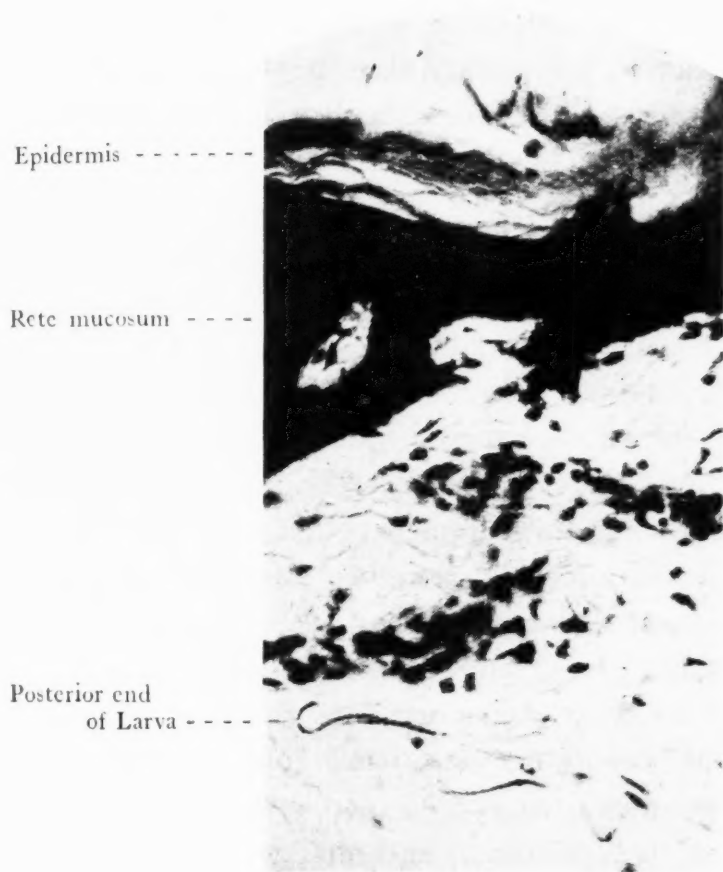


FIG. 2.—Photo-micrograph of section of skin to show the position of the larvae.

together with a drop or two of the saline solution from the bottom of the tube, the preparation fixed by heating, dried, and stained with haemalum. In no case was there obvious blood in the specimen.

The result of this examination was that filarial larvae of the species here described were found in twenty-two of the men (equal to 44 per cent.). It may be noted, moreover, that larvae of

*O. volvulus* were found in seventeen (equal to 34 per cent.), and that in eight of these cases the other larva was also present.

PERIODICITY. Ten of the men referred to above, in whom the larvae had been found, were re-examined two days later at about 9 p.m. No sensible difference suggesting periodicity in the prevalence of the larvae in the skin was observed.

DIAGNOSIS. The larva may be distinguished at a glance from that of *O. volvulus*, which also occurs commonly in the skin, by its slender body, crook-shaped posterior extremity, and blunt tail. In some respects it resembles the larva of *Filaria demarquayi*, but, apart from the fact that it apparently does not occur in the blood, it differs in that the tail is blunt, not sharply pointed, and that the column of nuclei extends practically to the tip of the tail.

The larva from which it has to be distinguished most carefully is that of *Ac. perstans*, which also is sheathless and striated and has a stumpy tail to the tip of which the column of nuclei extends, and which occurs in the blood, but may also be found in small pieces of skin removed in the manner described. The descriptions of the larva of *Ac. perstans* which we have been able to find are somewhat meagre, and do not agree in every respect. For example, Stephens (1916) gives the following measurements, length  $160\mu$  to  $210\mu$ , breadth  $5\mu$  to  $6\mu$ , nerve ring  $34\mu$ , excretory pore  $49\mu$ , genital pore  $125\mu$ , and notes that smaller larvae occur measuring  $90\mu$  to  $110\mu$  by  $4\mu$ ; Rousseau (1919) gives, length  $145\mu$  to  $185\mu$ , breadth  $3.5\mu$  to  $5\mu$ , nerve ring 25 per cent., excretory pore 32 per cent., G 1 cell 60 per cent., and anal pore 84 per cent.; and Johnston (1914) gives, length  $83\mu$  to  $170\mu$ , nerve ring 23.2 per cent., excretory pore 32.9 per cent., G 1 cell 62.6 per cent., and anal pore 83.5 per cent.

In order to obtain comparable data, twenty larvae of *Ac. perstans*, fixed and stained in the same manner as the other larvae, were measured by us. In these specimens the length varied from  $158\mu$  to  $214\mu$ , average  $179.4\mu$ , breadth  $2.5\mu$  to  $5\mu$ , and the approximate positions of the nerve ring, excretory pore, G 1 cell, and anal pore in a larva of the average length ( $179.4\mu$ ) were respectively 22.5, 32.7, 62.3, and 81.1 per cent. of the length from the anterior extremity.

The larva of *Ac. perstans* is, therefore, shorter than the larva described in this paper, relatively stouter, and the nerve ring, the

G 1 cell, and the anal pore are situated more anteriorly. When fixed in the manner described, it is, moreover, straight and not crook-shaped at its posterior extremity, and the column of nuclei at the anterior end is not reduced to a row of ten to twelve nuclei in single file.

For the new parasite we propose the name *Agamofilaria streptocerca*.

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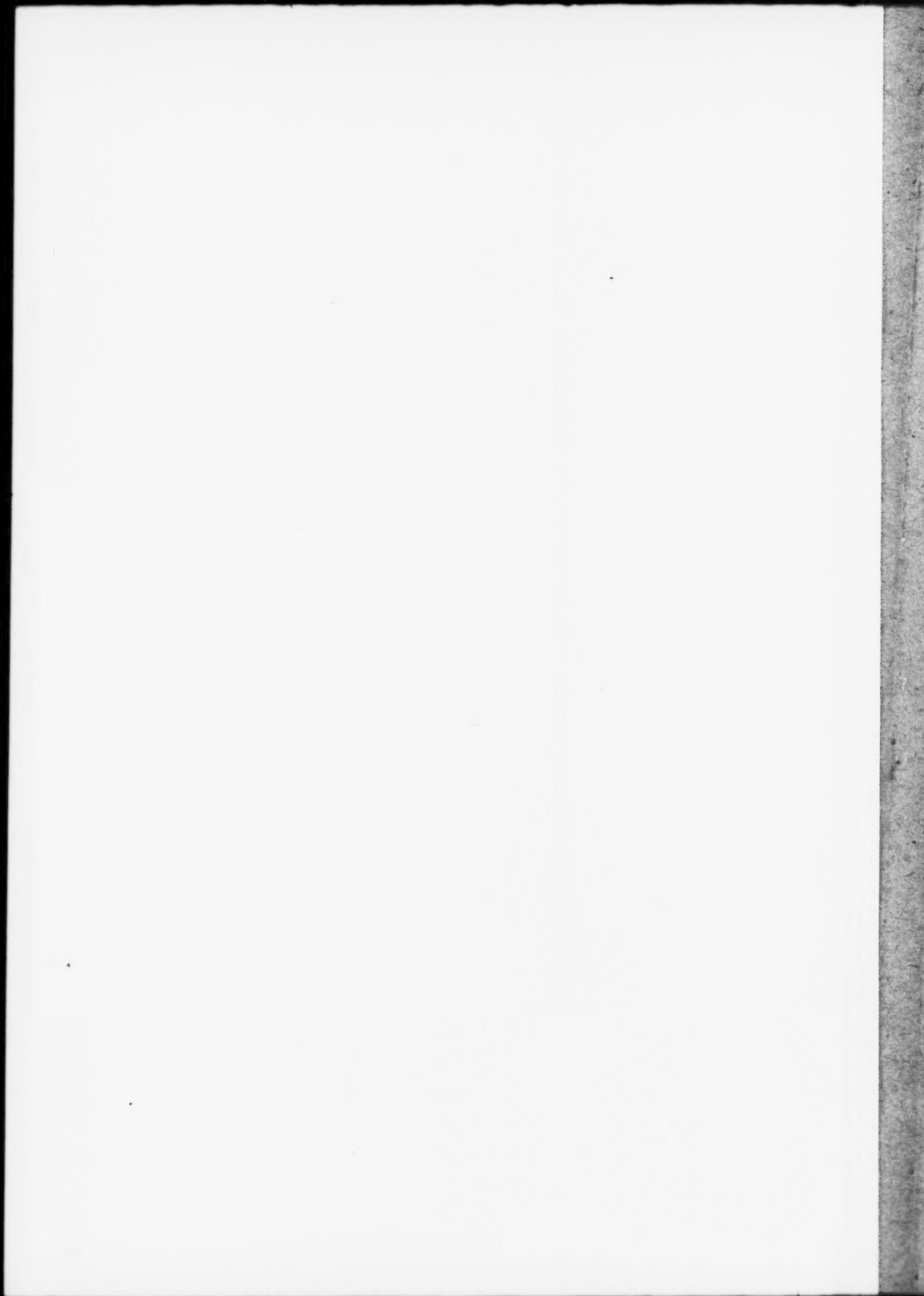
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# A CASE OF CREEPING ERUPTION IN A EUROPEAN IN THE GOLD COAST

BY

J. F. CORSON

(Received for publication 14 November, 1922)

## PLATE I

This skin affection is stated by Crocker (1903) and by Castellani and Chalmers (1919) to have been first described by Robert Lee in 1875; according to Roubaud (1914) the disease was observed in Norway by Hoegh in 1869, while Abraham, in a review of a paper by Knowles (1916), said that cases were recorded in Edinburgh 'more than sixty years ago,' i.e., before 1856.

When a cause has been found, it has usually been a larva of a fly of the family *Oestridae*, particularly *Gastrophilus* and *Hypoderma*. Castellani and Chalmers (*loc. cit.*) state that larvae of *G. haemorrhoidalis* and *G. nasalis*, of *Oestromyia satyrus* and of *H. bovis* and *H. lineata* have been found. Looss said that larvae of *Ancylostoma duodenale* in their passage through the skin could cause it. Sakurane (1917) found a *Ligula* parasite in a swelling of the skin, and suggested that the parasite of creeping eruption is of this nature. Ikegami (1919) removed from a case of this disease a young worm, said to have been probably *Echinorhynchus sphaerocephalus*, but a structure in it like an alimentary canal suggested *Gnathostoma*. Tamura (1919) removed a male *Gnathostoma* resembling *G. siamense*.

The disease is reported to have occurred in Ireland, Scotland, the Shetland Isles, Norway, Sweden, Denmark, Russia and Siberia, Bulgaria, Arabia, Sumatra, China, Japan, the United States of America, Brazil and West Africa (Senegal, Sierra Leone, Liberia, Togoland, Nigeria and the Cameroons).

The form of the disease occurring in Senegal, called locally Oerbiss or Lerbish, and for which no cause has been found, is considered by Roubaud to be of different aetiology from cases due to myiasis.

The following case showed a close resemblance clinically to the description of Oerbiss given by Roubaud.

81113  
A-24 24

Mr. G., British, living at Seccondee, Gold Coast, noticed, about the 18th of June, 1922, a small itching spot on the ball of the left thumb, and thought that it was probably due to a bite of some insect. A few days afterwards he noticed that the spot had become a line, and by the 26th of June there was a curved, raised, blister-like line about three-quarter inch long and one-sixteenth inch in diameter. Itching and a burning sensation were considerable, especially at night. On the 6th of July the appearance was as shown in Plate I. From then onwards until the beginning of October, when opportunities of observing the case ceased, the track progressed irregularly and intermittently round and along the thumb to near the tip. No parasite was found; microscopic examinations of serum and blood taken from various parts of the track and attempts at culture in broth and on agar and blood serum were without result.

No serious attempt to cure the disease was made; an ointment of sulphur and ammoniated mercury was used by the patient, who also opened the tracks from time to time and rubbed in tincture of iodine with apparent temporary benefit.

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EXPLANATION OF PLATE I

Case of Creeping Eruption.





# DEPTH, AND THE LARVAE AND PUPAE OF *STEGOMYIA FASCIATA*, F.

BY

J. W. S. MACFIE

(Received for publication 14 November, 1922)

The larvae of *Stegomyia fasciata*, as is well known, are usually found in small collections of water in domestic utensils, old tins, rot-holes in trees, calabashes, rock pools, etc.; they are to a large extent bottom- and side-feeders, and are capable of remaining, and in fact frequently do remain, completely submerged for very considerable lengths of time. In many tropical countries where *S. fasciata* is prevalent, water is stored in tanks of some size and depth. Even if efficiently screened, larvae may gain access to these tanks by being washed in with rainwater as eggs or young larvae, and it is a matter of some interest to know if, supposing they were introduced in this way, the larvae of *S. fasciata* would be able to thrive or would be likely to escape by being drawn off with water from a tap situated near the bottom.

Iyengar (1920) observed at Calcutta that larvae of *S. fasciata*, which are found there in almost all domestic situations in which larvae of *Anopheles stephensi* are found, are, however, rarely encountered with them in wells. He accounts for this difference in habit by the fact that whereas the larvae of *A. stephensi* are provided with hooks at the ends of the dorsal hairs on the ninth abdominal segment by means of which they cling to the sides, the larvae of *S. fasciata* lack these hooks, and, therefore, he assumes, were they to frequent wells would have to go to the bottom if the water was disturbed. 'It is likely,' he thinks, 'that mosquito larvae, being air-breathing organisms, cannot ordinarily stand much pressure at the depth of a well. *Stegomyia* is a bottom- and side-feeder; therefore it has to go to the bottom of its breeding-place, unlike *A. stephensi*, which feeds on the surface. These facts explain why *Stegomyia* has rarely been found in waters which are over three feet deep.'

These statements set us wondering if it was, indeed, the case that larvae of *S. fasciata* could not withstand the pressure of more than about three feet of water, and if disturbed must inevitably go to the bottom. The following experiments were carried out to ascertain the facts.

The apparatus used consisted simply of a wide-mouthed bottle with sloping shoulders, connected by a stout piece of rubber tubing with a length of wide-bore glass tubing. The tube and the bottle were set up vertically, the one above the other, and securely clamped. When required, additional lengths of tubing were added at the top with short rubber connexions.

In such a system larvae of *S. fasciata* lived apparently at ease, and after a day or two congregated at the top, mostly in the first foot, a few in the second, and only stray individuals at greater depths. The successive stages observed in an actual experiment are shown in the Table. As will be seen, the larvae, which at first were

Day	...	...	1	2	3	4	5	6
1st foot	...	..	+	+	++	++ (Mostly at the top)	++ (Nearly all at the top)	++ (Nearly all at the top)
2nd foot	...	...	+	+	++	+-	5	1
3rd foot	...	...	+	+	+-	o	1	o
4th foot	...	...	+	+-	1	o	o	o
5th foot	...	...	+	+-	o	o	o	o
6th foot	...	...	+	+-	o	o	o	o
7th foot	...	...	+	+	o	o	o	o

++ = many Larvae.    + = several Larvae.    +- = few Larvae.  
 +-- = very few Larvae.    1, 5, = one, five Larvae.

distributed throughout the tube, collected rather slowly at the top, so that after three days almost all of them were in the first foot of the column of water, and the majority at any particular moment actually at the surface. During this process of settling the habits of the larvae changed, bottom feeding being discontinued.

If then the tube was shaken or tapped, the larvae left the surface and wriggled down in the usual manner. They did not, however,



sink to the bottom; indeed, most of them descended only a few inches and very few more than one foot. Their descent was not passive, but was effected by active wriggling movements, and when these ceased they immediately began to float upwards towards the surface. Under ordinary circumstances, if disturbed the larvae wriggled downwards a few inches, ceased wriggling and floated upwards a short distance, and then recommenced active wriggling, this time towards the surface. They did not attempt to cling to the side of the tube. It is clear, therefore, that the larvae of *S. fasciata* when disturbed do not necessarily go to the bottom.

As the result of a single tap on the tube, it occasionally happened that one or two larvae descended to greater depths, such as two and a half feet or even three and a half feet. Larvae were also sometimes observed to descend voluntarily as much as five feet, and once one was found browsing on the side of the tube at a depth of 6 feet. By repeated tapping on the tube the larvae, could be urged to descend even deeper, eight feet at least. They did not appear to be at all incommoded by the pressure of the column of water, and when the tapping ceased wriggled back to the surface. Sometimes they rested on the bottom for a short time before starting the upward journey. It took one larva six minutes to regain the surface after descending seven feet.

In one experiment the system, consisting of the bottle and a long glass tube of wide bore of a total length of seven feet, was left standing until a copious growth of green algae had formed over the bottom, from which small bubbles of gas arose in sunlight and presumably kept the water oxygenated. In this system larvae of *S. fasciata* thrived better than they did when no algae were present, and were more frequently seen at greater depths; indeed, both young and older larvae, but especially the former, were often seen browsing actually on the bottom. The pressure of the seven-foot column of water above them appeared to have no injurious effect whatsoever.

The pupae of *S. fasciata*, however, are not able to descend unharmed to such great depths as the larvae. As the result of a single tap on the tube, they usually descended only an inch or two and then floated passively back to the surface. By repeated tapping they could be induced to descend considerably further, but beyond

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Day	...	...	1	2	3	4	5	6
1st foot	...	..	+	+	++	++ (Mostly at the top)	++ (Nearly all at the top)	++ (Nearly all at the top)
2nd foot	...	...	+	+	++	+-	5	1
3rd foot	...	...	+	+	+-	o	1	o
4th foot	...	...	+	+-	1	o	o	o
5th foot	...	...	+	+-	o	o	o	o
6th foot	...	...	+	+-	o	o	o	o
7th foot	...	...	+	+	o	o	o	o

++ = many Larvae.    + = several Larvae.    +- = few Larvae.

+-- = very few Larvae.    1, 5, = one, five Larvae.

distributed throughout the tube, collected rather slowly at the top, so that after three days almost all of them were in the first foot of the column of water, and the majority at any particular moment actually at the surface. During this process of settling the habits of the larvae changed, bottom feeding being discontinued.

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In one experiment the system, consisting of the bottle and a long glass tube of wide bore of a total length of seven feet, was left standing until a copious growth of green algae had formed over the bottom, from which small bubbles of gas arose in sunlight and presumably kept the water oxygenated. In this system larvae of *S. fasciata* thrived better than they did when no algae were present, and were more frequently seen at greater depths; indeed, both young and older larvae, but especially the former, were often seen browsing actually on the bottom. The pressure of the seven-foot column of water above them appeared to have no injurious effect whatsoever.

The pupae of *S. fasciata*, however, are not able to descend unharmed to such great depths as the larvae. As the result of a single tap on the tube, they usually descended only an inch or two and then floated passively back to the surface. By repeated tapping they could be induced to descend considerably further, but beyond

a certain depth (which in our experiments appeared to be about three feet to three and a half feet) they showed an unquestionable anxiety to return to the surface, ceasing to respond readily to disturbances, such as tapping or shaking, even when violently applied, descending further only very reluctantly, and sometimes refusing to move at all or actually ascending in spite of everything. In one experiment, by means of repeated tapping and shaking, a pupa was driven down to the bottom, a distance of seven feet. From this position it struggled upwards, evidently with increasing difficulty, for a distance of a little more than four feet. At about this level it managed to maintain itself for several minutes, now jerking itself up an inch or so, now sinking an inch or so, and then began to lose ground, at first slowly, then more quickly, and eventually sank to the bottom. Another pupa was similarly induced to descend five feet, but it managed to regain the surface. The inability of pupae to descend without ill-effects to such great depths as the larvae appeared to be dependent on their diminished buoyancy at such depths, which caused them to begin to sink the moment active movement was arrested. This fact should be correlated with the imperative need of pupae of access to air, for the strenuous efforts exerted in struggling upwards from an unaccustomed depth no doubt accelerated the exhaustion of the supply of air in their tracheal tubes.

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# A NOTE ON THE ACTION OF LITHIUM CHLORIDE ON MOSQUITO LARVAE

BY  
J. W. S. MACFIE

(Received for publication 14 November, 1922)

It is well known that larvae of certain mosquitoes, e.g., *Stegomyia fasciata*, are intolerant of common salt. Other chlorides act similarly, some of them very powerfully, as is shown in Table I,

TABLE I.

The number of hours required to kill all larvae of *Stegomyia fasciata* in various solutions of chlorides.

Salt	Percentage of anhydrous salt which = 1.0% Cl	Cl 1.0%	Cl 0.75%	Cl 0.5%	Cl 0.25%
ZnCl <sub>2</sub> ... ..	1.92	3 hours	3 hours	7 hours	7 hours
BaCl <sub>2</sub> ... ..	2.93	4 hours	5 hours	<24 hours	<24 hours
LiCl ... ..	1.21	5 hours	7 hours	>7 hours	<24 hours
NaCl ... ..	1.65	6 hours	<24 hours	>48 hours	>72 hours
CaCl <sub>2</sub> ... ..	1.56	6 hours	>24 hours	>48 hours	>72 hours
MgCl <sub>2</sub> ... ..	1.34	<24 hours	>24 hours	>48 hours	>72 hours

which summarises a series of preliminary experiments on the action of these salts carried out by Mr. R. Simmons, which I am permitted by him to quote. Additional experiments were made subsequently with lithium chloride and *Stegomyia fasciata*. In one of these, five larvae and one pupa were placed in a 1.2 per cent. solution of LiCl; within four hours all the larvae were dead, but the pupa appeared to be unaffected. In three others, twenty-seven larvae were placed in a 0.3 per cent. solution of LiCl in the afternoon; all were dead by next morning, that is within sixteen or seventeen hours.



During the experiments it was noted that lithium chloride not only killed the larvae of *Stegomyia fasciata*, but also produced a peculiar effect on them, causing them to writhe about at the bottom of the jars, apparently unable to rise to the surface, and to become entangled with one another, usually by the mouth brushes. These effects were observed even in the weakest solutions used.

As lithium chloride appeared to have a very powerful effect on the larvae, further experiments were carried out to determine the limits of the injurious action.

*Culex fatigans*. The larvae were placed in glass jars (five in each) containing 100 c.c of the lithium chloride solution. The jars were covered with glass plates, stood on the laboratory bench, and examined morning and afternoon at about 9 a.m. and 5 p.m. The solutions used were 0.3, 0.15, 0.06, 0.03, and 0.015 LiCl per cent. The results are summarised in Table II.

TABLE II.

The effect of solutions of Lithium chloride on the larvae of *Culex fatigans*.

Day of the experiment	Percentages of LiCl in the solutions				
	0.3	0.15	0.06	0.03	0.015
1. a.m.	Experiments started				
p.m.	Three dead	All affected	One affected	No visible effect	No visible effect
2. a.m.	All dead	All dead	Three affected	No visible effect	No visible effect
p.m.	—	—	All sluggish	No visible effect	No visible effect
3. a.m.	—	—	Three almost dead	No visible effect	No visible effect
p.m.	—	—	Two just alive	No visible effect	No visible effect
4. a.m.	—	—	—	No visible effect	No visible effect
p.m.	—	—	One alive	No visible effect	No visible effect
5. a.m.	—	—	All dead	No visible effect	No visible effect
p.m.	—	—	—	No visible effect	No visible effect

Exactly similar experiments were carried out with larvae of *Stegomyia fasciata* and *Anopheles costalis*. Without entering into details, it may be said that the results also were similar, all, or practically all, the larvae dying in the 0.3 and 0.15 per cent. solutions within twenty-four hours, and in the 0.06 per cent. solution

within two or three days. In the 0.03 and 0.015 per cent. solutions the larvae, especially when young, were also affected: in an experiment with almost fully grown larvae of *S. fasciata*, for example, only two out of ten completed their development in the former solution, and two out of seven in the latter, whereas in the control jar no casualties occurred. In the case of *S. fasciata*, entanglement of the larvae by their mouth brushes and other setae was repeatedly, but not invariably, observed.

It is worthy of note that the larvae of *Mansonioides africanus*, which live attached to the roots of the water-weed *Pistia stratiotes*, do not escape the action of lithium chloride. A small plant of *Pistia stratiotes* with larvae attached to it was placed one afternoon in a jar containing 100 c.c. of a 0.3 per cent. solution. By the next morning, that is within eighteen hours, all the larvae had left the roots of the plant and were dead.



# MALARIA IN CHIMPANZEES IN SIERRA LEONE

BY

S. ADLER

(Received for publication 15 November, 1922)

## PLATES II AND III

Reichenow (1920) working in the Cameroons, found parasites indistinguishable from human malaria parasites in the blood of gorillas and chimpanzees. Of eight chimpanzees examined six were found to be infected, one with *Plasmodium vivax* ? forms (gametocytes only), two with *Plasmodium falciparum* forms (crescents only), one with *P. falciparum* and *P. vivax* forms, and two with *P. falciparum* and *P. vivax* forms together with *P. malariae* forms.

Reichenow found that infections were heaviest in young animals, and suggested that resistance is acquired after attacks in early life.

Blacklock and Adler (1922), of the Liverpool School of Tropical Medicine, described a parasite resembling *Plasmodium falciparum* in a chimpanzee, and forms resembling *P. vivax* and *P. malariae* also occurred, but the only form of gametocyte found was the crescent.

I have recently examined thirteen additional chimpanzees, six of which were caught near Pendembu, and six near Blama, in the Sierra Leone Protectorate, and one from an unknown locality.

Of these thirteen animals, two were found to be infected with parasites indistinguishable from *P. falciparum*. The infected cells were not enlarged or pale, and many of the delicate rings showed two bars of chromatin. In both cases crescents were found, but only after prolonged search, resembling in this respect human infections with *P. falciparum* in West Africa. Parasites resembling simple tertian or quartan forms were not found.

CASE I. Captured near Blama. The animal was emaciated and weak. A blood examination on 8th September, 1922, showed numerous rings and a few crescents. On 9th September, 1922, quinine hydrochloride, 0.5 grains, was administered intramuscularly; rings were present in the blood until 14th September, 1922, but crescents persisted until the animal's death on 2nd October, 1922.

The animal's condition showed no marked improvement after the disappearance of rings from the peripheral blood; its appetite was poor and it often passed loose stools containing a large amount of fat globules. Death occurred after an attack of enteritis, which was apparently caused by an invasion of *Oxyuris* sp., of which large numbers (all immature) were passed in the animal's stool.

Post-mortem, malaria pigment was found in the spleen, liver, and bone marrow, and crescents, in small numbers, in the bone marrow. The liver showed fatty changes. Enormous numbers of immature *Oxyuris* were found in the large intestine.

25th September, 1922. Advantage was taken of the fact that rings had not been seen in the blood for eleven days, and that crescents still persisted, to test the theory of parthenogenesis. 0.4 c.c. of the animal's blood were injected into another chimpanzee in which malaria parasites had never been found since it first came under observation on 4th September, 1922.

The injected animal was observed till the 11th November, 1922, but parasites were not found in the peripheral blood.

CASE II. Captured near Pendembu. The animal was extremely emaciated. On 12th September, 1922, rings and crescents were found, but the infection gradually disappeared without treatment, and on the 19th September, 1922, the blood became negative. The animal's condition gradually became worse, it took very little food, the stools were loose and always contained fat globules.

On 30th October, 1922, the blood examination again showed a few ring-form parasites.

The animal died on 30th October, 1922. Post-mortem pigment was found in the spleen and bone marrow, and a small number of schizonts in the spleen; no crescents were found. The liver was pale, and on section showed extreme fatty degeneration and infiltration, the majority of the liver cells being destroyed.

It is interesting to note that both animals were young (under two years). Older animals, including one old adult, were negative. This supports Reichenow's suggestion that in chimpanzees, as in natives, resistance is acquired after attacks in early life.

#### THE RELATIONSHIP OF MALARIA IN CHIMPANZEES TO HUMAN MALARIA IN SIERRA LEONE

Although the malaria parasite in the chimpanzee in Sierra Leone is morphologically indistinguishable from *P. falciparum*, there is as yet no evidence that it is this species.



Mesnil (1920) failed to infect a chimpanzee by intravenous injections of human blood infected with *P. falciparum*. He also failed to infect the same chimpanzee by the bite of Anophelines with sporozoites of *Plasmodium falciparum* in their salivary glands.

Blacklock and Adler (1922) failed to infect:—

(1) Two Europeans by intravenous and subcutaneous injections of heavily infected blood from a chimpanzee.

(2) A chimpanzee by an injection of 3 c.c. of blood heavily infected with *P. falciparum* from a patient during his first attack of malaria, which he acquired in Sierra Leone.

(3) *Anopheles costalis* by feeding on a chimpanzee; but it should be noted that crescents were scanty in the animal's blood.

The existence of a relationship between human malaria and malaria in chimpanzees cannot be conclusively proved or disproved, until the insect vector of the latter be discovered and experiments with the infective vector carried out on human beings.

My best thanks are due to Mr. W. Addison, Provincial Commissioner of Kennema, and Mr. N. C. Hollins, District Commissioner of Pendembu, through whose kindness I obtained a number of chimpanzees.

### SUMMARY AND CONCLUSIONS

Thirteen chimpanzees were examined for malaria in Sierra Leone.

Two young animals were found to be infected with a parasite indistinguishable from *P. falciparum*.

Older animals were negative, and resistance following attacks in early life is, therefore, suggested.

Blood from one chimpanzee containing only crescents failed to infect another chimpanzee.

Both infected animals on post-mortem examination showed fatty changes in the liver.

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EXPLANATION OF PLATE II

Malaria Parasites.

Figs. 1 to 17. Ring forms.

Figs. 18 to 19. Crescents.



1



2



3



4



5



6



7



8



9



10



11



12



13



14



15



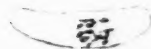
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20  $\mu$

## EXPLANATION OF PLATE III

- Fig. 1. Micro-photograph of liver (Case II), showing fatty degeneration and infiltration.  $\times 250$ .
- Fig. 2. On the right, young chimpanzee with malaria, showing emaciation. (Note absence of paunch.)  
On the left, healthy young animal.

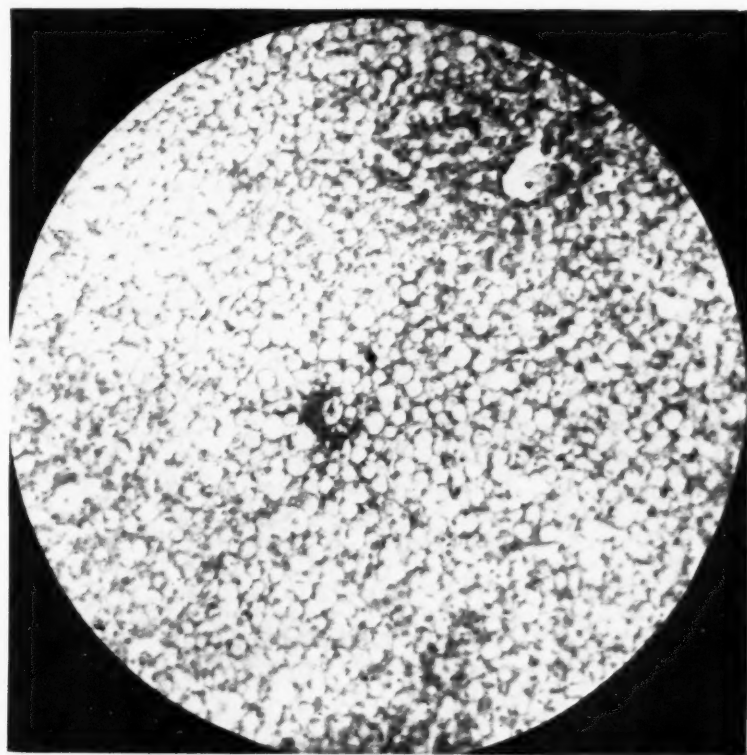


FIG. 1

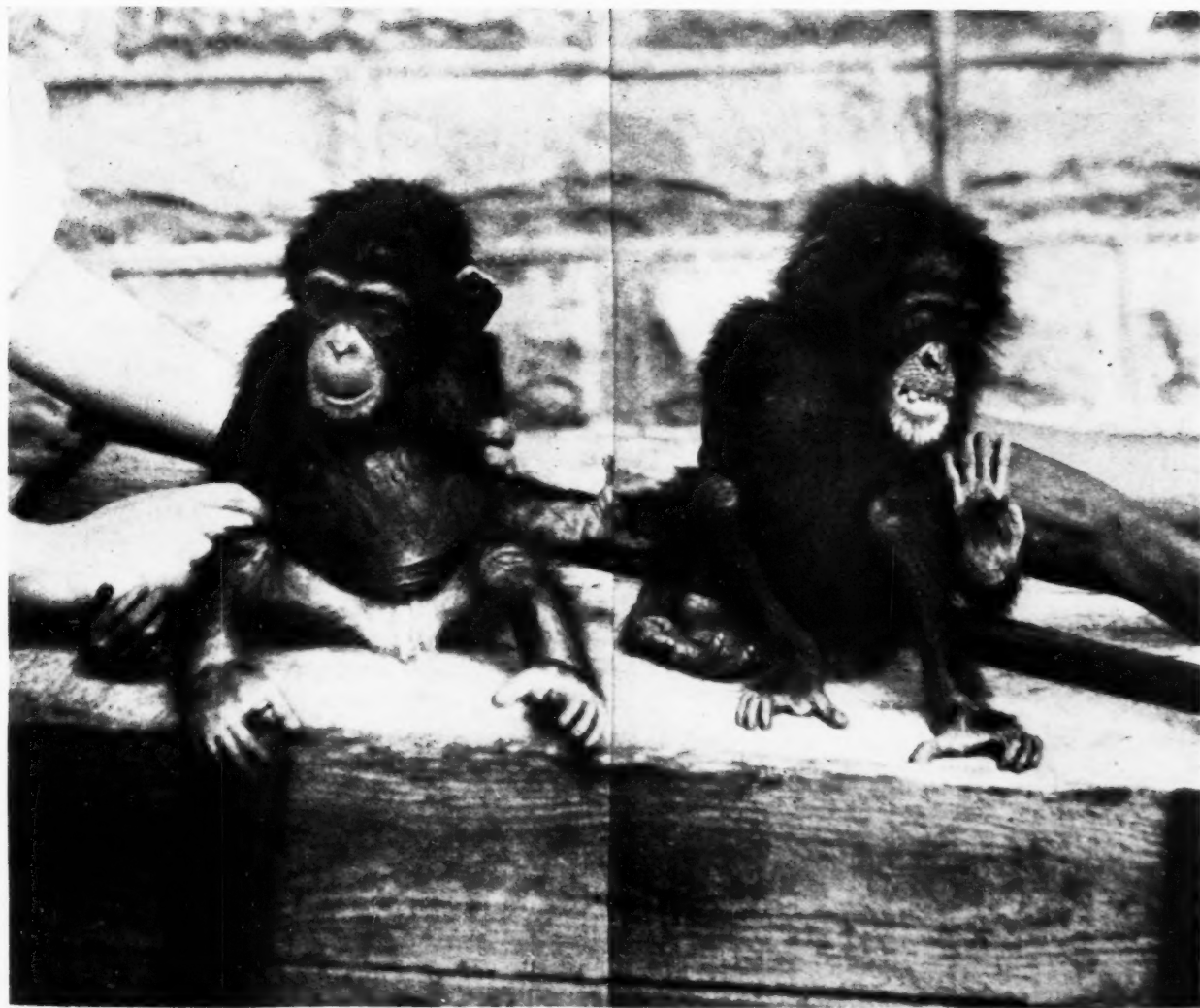


FIG. 2



A

# NEW AND OLD OBSERVATIONS ON CERATOPOGONINE MIDGES ATTACKING OTHER INSECTS

BY

F. W. EDWARDS

*(Published by permission of the Trustees of the British  
Museum)*

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During the last two decades a fairly extensive literature has been published in regard to the attacks made by Ceratopogonine midges upon other insects. Several cases were placed on record by Knab (1914), who also reviewed the literature published previously to this date. More recently the facts have been reviewed by Peyerimhoff (1917) and Kieffer (1922), the former author adding some very interesting observations of his own. There are, however, one or two additional and very interesting cases which have been overlooked by all the above-named writers, and also some further unpublished instances which have come to my notice. It may, therefore, be worth while, in recording the fresh cases, to review again the literature of the subject.

The attacks made by midges upon other insects fall under two main heads:—

(1) *Cases of predacity*, where the midges attack other adult insects of approximately their own size, or not much larger, and kill them by puncturing their skin and sucking them dry. A good many instances of this have been noted, and I have summarised them in a recent paper (1920).

The following list gives the names of these species and of their victims :—

PREDATOR	VICTIM
	<i>NEUROPTERA</i>
<i>Palpomyia flavipes</i> , (Mg.)	Ephemerid ( <i>Baetis</i> sp.)
" sp.	Perlid
	<i>CHIRONOMIDAE</i>
<i>Bezzia annulipes</i> (Mg.)	<i>Tanytarsus sylvaticus</i> , v.d. Wulp.
<i>Probezzia multiannulata</i> (Strobl.)	<i>Culicoides circumscriptus</i> , Kieffer.
<i>Probezzia</i> ? <i>signata</i> (Mg.)	<i>Culicoides pulicaris</i> (L.)
<i>Stilobezzia gracilis</i> (Hal.)	<i>Cricotopus pulchripes</i> , Verr.
" "	<i>Orthocladius</i> sp.
" "	<i>Tanytarsus</i> , 2 spp.
" "	<i>Tanytus binotatus</i> , Mg.
<i>Serromyia femorata</i> (F.)	<i>Cricotopus pulchripes</i> , Verr.
" "	<i>Bezzia ornata</i> (Mg.)
" "	<i>Serromyia femorata</i> (F.) ♂
* " "	<i>Trichocladius</i> sp.
† <i>Ceratopogon candidatus</i> , Winn.	<i>Trichocladius</i> sp.
<i>Ceratopogon lacteipennis</i> , Zett.	<i>Camptocladius</i> ? <i>gracilis</i> , Goet.
" "	<i>Culicoides arcuatus</i> (Winn.)
" "	<i>Ceratopogon lacteipennis</i> Zett., ♂

In addition, Kieffer (1922) quotes Loew to the effect that *Macropheza albitarsis*, Mg., preys upon other small insects.

This list could no doubt be greatly extended by careful observation, and it seems probable that all the members of the bare-winged genera of *Ceratopogoninae* are normally predaceous in the female sex. Evidently the various modifications of the legs, such as swollen and often spiny femora, enlarged claws and spines on the last tarsal segments, which the females of most of these genera exhibit, are to be regarded as adaptations for holding their insect prey. It is probable that these predaceous habits are primitive in this sub-family, and that they have directly or indirectly led to the more specialised blood-sucking habits of certain species and genera.

Although it is beyond the scope of this paper, attention may be called in passing to the observations of Ingram, who found in West

\* Noted in North Cornwall, June, 1922. This is the only fresh record I have to add to the list previously published.

† Goetghebuer's review (1922) of the *Ceratopogoninae* in Meigen's collection has made it clear that *C. communis*, Mg., the type of the genus, belongs to Kieffer's genus *Psilobealea*; this name, therefore, falls as a synonym of *Ceratopogon*. As I have stated in a recent paper (1921) I do not consider the differences between *Ceratopogon* (*Psilobealea*) and *Isobealea* are of more than subgeneric value, hence I include *I. lacteipennis* and its allies also in *Ceratopogon*.

Africa the larvae of *Forcipomyia ingrani*, Carter (1919), attacking mosquito larvae. This is, I believe, the only known instance of predacity in a *Ceratopogonine* larva.

(2) *Cases of blood-sucking*, where the attacking midge sucks the juices of its victim, without as a rule killing it, the victim in such cases being generally much larger than the attacking species. It is this class of phenomena with which I wish to deal more particularly in the present paper. Following the example of Peyerimhoff (1917), we may consider these midges in several groups, according to the type of host which they attack.

### I. SPECIES ATTACKING MOSQUITOES

A considerable number of observations have been made on the relations between adult mosquitoes (generally *Anopheles*) and a species of *Culicoides* which is widely spread in the Oriental region. In a recent paper (1922) I have summarised these observations, and have described the midge concerned as *Culicoides anophelis*. It appears that the object of the *Culicoides* is to obtain engorged blood from the abdomen of its host, though it has in some cases been found to have attacked mosquitoes which were not engorged. At present only this single species of *Culicoides* is known to have these very remarkable habits.

This extremely interesting case may be regarded in one of two ways. It may be a development directly from a primitive predacity; the species having passed from a diet of (say) *Chironomidae* to one of mosquitoes, and thence to the mammalian blood contained in the body of its host. In this case it is easy to imagine that the midge might follow its mosquito host to its feeding ground, and eventually take to sucking blood itself directly from the mammal, thus giving rise to the blood-sucking habits now so general in the genus *Culicoides*. The possibility of this having been the course of development is somewhat strengthened by the fact that *C. anophelis* appears to show some somewhat primitive characters, such as the simple wing-pattern and rather large radial cells. On the other hand, it may be that the habit of obtaining blood from mosquitoes is purely secondary, and derives from an ordinary direct method of blood-sucking; this is, perhaps, most probable, since Lamborn's observations seemed to show that a blood meal was essential to the production of a complete fertile batch of eggs.

## II. SPECIES ATTACKING ADULT LEPIDOPTERA

One instance has been recorded (by Kryger, 1914, quoted also by Knab, 1914) of a midge attacking a moth. The host was *Cidaria didymata*, L.; the midge was not precisely identified, but was stated by Knab to be apparently an undescribed species, 'belonging in the neighbourhood of *Ceratopogon murinus*, Winn.' In the hope of obtaining some further information concerning this species, I wrote to Mr. Kryger in Denmark, and also to Messrs. Aldrich and Böving in Washington, but only to discover that the material had been lost.

A second very similar case was discovered by Professor Newstead in North Wales in 1914, and I am greatly indebted to him for kindly allowing me to examine and describe the material of this most interesting find. While collecting at night with the aid of an acetylene lamp, Professor Newstead came across a cabbage-white butterfly whose wings were being attacked by nine specimens of a *Ceratopogonine* midge. The butterfly was considerably damaged, and as is shown by the accompanying photograph (fig. 1) the

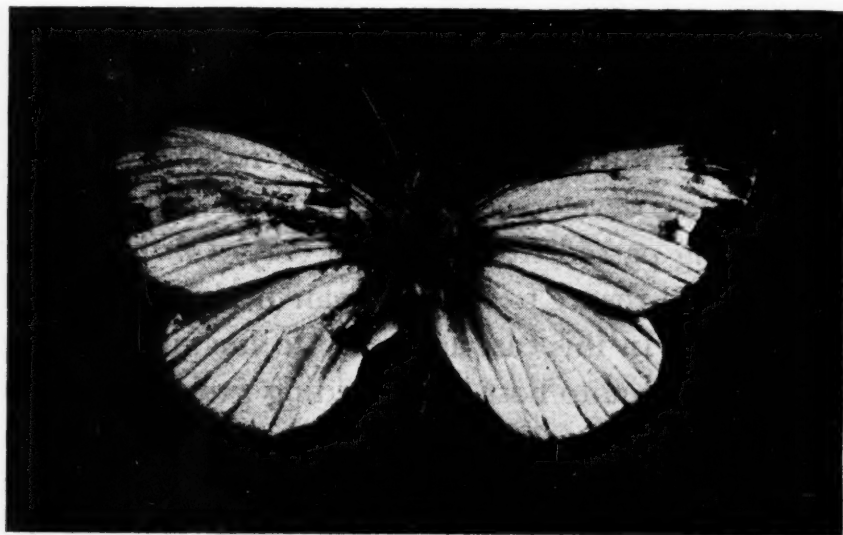


FIG. 1. *Pieris napi* (slightly enlarged), victim of *Forcipomyia* (*Euforcipomyia*) *papilionivora*, Edwards. The left forewing, just below the costa and along both sides of the large vein, shows the nature of the damage, caused by the midges

damage would seem to have been caused, at least in part, by the attacks of the midges.\* The latter appeared to be eating the wings

\* Blood was seen exuding from the ruptured veins when the insect was captured; and the scales on either side of the veins are stained russet-brown, due apparently to the exudation. When first imprisoned the midges left their victim and swarmed over the glass lid of the collecting box; but on placing them in the dark, they were found, two hours later, to have resumed their attacks on the butterfly. (R. Newstead).



of the butterfly, though, as in the case of the Danish insects, they may in reality have been sucking juices from the wing-veins, especially if the blood was exuding from the broken ends of the veins.

After a careful examination of the literature, I have come to the conclusion that the midges collected by Professor Newstead belong to an undescribed species, and I, therefore, name and describe it as follows:—

*Forcipomyia (Euforcipomyia) papilionivora*, sp. n.

*Head* rather densely clothed with golden pubescence. Eyes practically touching, perfectly bare. *Antennae* uniformly dark, flagellum clothed with longish dark hair, nearly twice as long as the diameter of the segments. First eight flagellar segments together much shorter than the last five together (proportions 3 : 5). First flagellar segment nearly globular, the next five slightly transverse, seven and eight again practically globular, nine to thirteen each nearly three times as long as broad, thirteen with a nearly globular, nipple-like tip; one to eight each with rather long and stout sense-bristles, difficult to observe. *Palpi* dark, the second segment oval, broadest in the middle, not quite twice as long as its greatest breadth, apical part not suddenly narrowed; last two segments together as long as the second, the fourth a little longer than the third. Second segment with a globular internal cavity opening by a small round pore on the inner face. *Mandibles* broad, about three to five times as long as their greatest breadth, tip rather bluntly rounded, with about twelve to fifteen small, equal-sized teeth on one side; in the middle is an oval clear spot enclosing an elongate dark mark, resembling that figured by Carter, Ingram and Macfie in the genus *Prionognathus*. *Maxillae* almost as long as the mandibles, with about twenty-five fine regular crenulations, scarcely teeth, on one margin. *Hypopharynx* rather elongate, oval, a little over twice as long as broad, tip smooth. *Thorax* with the integument dull blackish, the humeral angles and the whole scutellum dull yellow. *Mesonotum* densely covered with short, bright golden pubescence mixed with longer, but not very long, brownish hair. Scutellum similarly but less densely clothed, postnotum shining black. *Abdomen* rather narrow for the genus, dull dark brown, uniformly clothed with short blackish hair. *Spermathecae* large,

nearly globular, necks practically without chitination. Cerci dark. *Legs* slender, practically uniform in colour, rather dark brownish, very hairy, the tibiae with some long hairs which are about six times as long as the tibial diameter. On all the legs the first tarsal segment is 2.5 times as long as the second. Empodia well developed, almost as long as the claws. *Wings* clothed rather densely (but somewhat less densely than in most species of the genus) with close-lying dark hair; most of the hair on the thick veins golden, but mixed with some dark. Venation normal for the genus: *Rs* in contact with *R*<sub>1</sub>, so that the first radial cell is obliterated; second radial cell about twice as long as broad, trapezoidal; petiole of median fork a little shorter than the very oblique r-m; cubitus forking below end of costa, which reaches just beyond the middle of the wing. *Halteres* with the stem dark, the knob white.

Length of body, 1.8 mm.; wing, 1.4 mm.

NORTH WALES: Ty Gwyn Farm, Aberhosan, Machynlleth, found at 10.15 p.m. feeding on the wings of *Pieris napi* (R. Newstead). Three ♀ co-types in the British Museum, presented by the collector; six others in the collection of the Liverpool School of Tropical Medicine.

This insect seems to have no very close ally among the European species. By Kieffer's table it will run down to *F. formicaria* (Kieff.), which differs in having the first tarsal segments much shorter, as well as in the palpal structure and other details. Other European species which show some points of resemblance are *F. hirta* (Lundst.) and *F. murina* (Winn.), but none show the combination of antennal and tarsal characters possessed by this species. In both these respects the new species resembles *Lasiohelea velox* (Winn.), but it has not the venation of the genus *Lasiohelea*; it belongs to Malloch's group *Euforcipomyia*, and bears a close resemblance to the North American *E. fusicornis* (Coquillett) (see below).

From the above remarks it will be seen that the specimens collected by Professor Newstead might have been referred to as 'an undescribed species belonging in the neighbourhood of *Ceratopogon murinus*, Winn.,' and it, therefore, seems not improbable that the Danish specimens found by Mr. Kryger may have belonged to the same species. In any case, it is interesting

to note that the only two records we have of midges attacking adult *Lepidoptera* both refer to an insect belonging to the same group of the genus *Forcipomyia*.

### III. A SPECIES ATTACKING A SIALID

Malloch (1915) states that he has seen a specimen of *Euforciomyia fusicornis* (Coquillett) which was taken attacking a Sialid (*Chauliodes* sp.). In view of the large size of the victim, this must be classed as a case of blood-sucking rather than of predacity. It is not stated what part of the Sialid was attacked, but it is evident that we are here dealing with a very similar case to the two last considered, *Chauliodes* being a large-winged, rather soft-bodied insect, comparable with a moth. It is, therefore, of special interest to note that *E. fusicornis*, according to Malloch's description, bears a very close resemblance to the species just described as *F. papilionivora*. In fact, it is not impossible that the two may be conspecific, though it seems unsafe to identify a European with a North American form without actual comparison of material.

Although I do not consider *Euforciomyia* to be generically distinct, it may be retained as a sub-genus in the sense in which Malloch proposed it: i.e., to include the species of *Forcipomyia* which have the first hind tarsal segment markedly longer than the second, reserving *Forcipomyia* (*s. str.*) for those species in which the first is shorter, or at most slightly longer, than the second. This is not the sense in which Kieffer has used the name, but seems to be the correct one, since the type species of *Forcipomyia* is *bipunctata*, L. (*trichoptera*, Mg.), not *albipennis*, Mg., as stated by Kieffer.

### IV. SPECIES ATTACKING CATERPILLARS

A number of cases of midges attacking caterpillars have been recorded from time to time. Most of these were referred to by Knab (1914), the cases he mentioned being as follows:—

SPECIES	HOST	OBSERVER
<i>Forcipomyia propinqua</i> (Will.)	<i>Melanchroia geometroides</i> (Waker) ( <i>Geometridae</i> )	Baker (Cuba)
<i>F. squamosa</i> , Lutz.*	Sphingid (undetermined)	Townsend (Peru)
<i>F. sp.</i>	Sphingid (undetermined)	Barbiellini (Brazil)
<i>F. crudelis</i> , Knab.†	Not stated	Urich (Mexico)
<i>F. erucicida</i> , Knab.	<i>Erinyis ello</i> L. ( <i>Sphingidae</i> )	Mosier (Florida)

\* Specific name given by Lutz (1914).

† This specific name is preoccupied by *F. crudelis* (Karsch), but I refrain from proposing a substitute because the descriptions appear to indicate that the species is almost certainly identical with *F. tropica*, described by Kieffer (1917) from Costa Rica.

All the above-named species of *Forcipomyia* belong to that group of the genus in which the female tibiae are devoid of scales, and the second segment of the hind tarsi is at least twice as long as the first. The same remark is true of three other species which were not known to Knab, and are discussed below. It would seem, therefore, that the habit of attacking caterpillars is a very special one, restricted to this group of the genus *Forcipomyia*.

*Forcipomyia crudelis* (Karsch, 1886)

This species was described from a single female found by Karsch sucking a saw-fly larva in the neighbourhood of Berlin. He remarks that his attention was called to the larva by the movements which it made in endeavouring to dislodge its tormentor, and that the latter had its mouth-parts so firmly fixed in the body of its victim that it did not loose its hold even when the pair were placed in the cyanide bottle. *F. crudelis* has not been recognised since Karsch described it, but it is evidently very closely related to *F. pallida* (Winn.), *F. brevimanus* (Lundst.) and *F. alboclavata* (Kieffer).

*Forcipomyia hirtipes* (de Meij.)

Two females of this species were found by Mr. J. C. F. Fryer (recorded by me, 1913), at Peradeniya, Ceylon, each sucking a larva of *Papilio clytia*. *F. hirtipes*, it is interesting to note, closely resembles the European *F. alboclavata*, showing only very slight differences in the proportions of the palpal and tarsal segments. The Ceylon specimens do not agree with de Meijere's description, as regards the middle tarsi; but, as he has informed me, the description is incorrect. In reality, he says 'the mid-tarsal segments have about the same proportions as the hind, viz., in ♂ about 8 : 30 : 14 : 11 : 8, in ♀ about 9 : 25 : 11 : 9 : 8.' He also informs me that though the antennae of the type ♀ are mutilated, in another specimen the proportion of the first eight to the last five flagellar segments is about 27 : 38. These proportions are about the same as in the Ceylon specimens.

*Forcipomyia alboclavata* (Kieffer, 1919), (*canaliculata*,  
Goetghebuer, 1920)

The British Museum possesses three ♀♀ of this species from Taterafuered, Hungary, 1906 (*Hon. N. C. Rothschild*), on which



the donor sent the following note:—‘Sitting on the backs of larvae of *Deilephila galii* (which were extremely common in a large field near Taterafuered), and appearing to eat some secretion from their skins.’ The specimens have the second hind tarsal segment 2.5 instead of only twice as long as the first, but otherwise agree with Keiffer’s description, and I have no hesitation in quoting the synonymy as above.

*F. alboclavata* has been found in Scotland (Arran) as well as Belgium, but its habits in these countries have not been observed.

#### V. SPECIES ATTACKING OIL-BEETLES

Peyerimhoff (1917) has given an interesting account of the relations between a Ceratopogonine midge (at present undetermined) and the oil-beetle *Meloe majalis*, L., in Algeria. The flies, he says, pursue these large beetles in little swarms, and without inconveniencing them in any way, feed upon their yellow blood. M. de Peyerimhoff informs me that the flies are now in the hands of Professor J. J. Kieffer, who believes that they represent a new species.\*

More recently, a second similar instance, this time from Denmark, has been recorded by Hansen (1921). I am indebted to my friend, Mr. J. P. Kryger, for the following translation of Mr. Hansen’s note:—

‘A gnat attacking a *Meloe*—29th May, 1921. I saw a *Meloe proscarabaeus* crawling along a walk in the wood of Ulvlyst (Denmark). A little swarm of gnats hovered over the beetle and sometimes attacked it, especially on the soft skin between the first and second thoracic segments. The beetle was seriously affected by the gnats biting, and rubbed its sides with its hind legs, but without getting rid of its tormentors. When I put the collecting bottle over the beetle two gnats were sitting on its back, but as it tumbled in twelve gnats appeared in the bottle. The remaining ten must have been sitting on the underside of the beetle.’

Mr. Kryger has further been so good as to obtain for me the loan of the specimens captured by Mr. Hansen, which had been

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\* Since this was written I have received, through the kindness of Professor M. Bezzi, a number of the specimens originally collected by M. de Peyerimhoff. Without trespassing on ground to be covered by Professor Kieffer, I may remark that these specimens represent a species which is extremely nearly related to *Atrichopogon rostratus* (Winn.), a fact which is of much interest in view of my determination of the Danish specimens.



presented by the collector to the Zoological Museum at Copenhagen. Upon examination of the flies I find that they belong to the species *Atrichopogon rostratus* (Winn.), all, of course, being females. The purpose of the formidable proboscis possessed by this species thus becomes apparent for the first time, for neither it nor any other member of its genus has been known either to bite warm-blooded animals or to prey upon other small insects. But, as in the case of many other midges with strong food preferences, the diet of *A. rostratus* is not confined to the blood of *Meloe*, but consists partly of vegetable substance (honey, or perhaps pollen). All the adult specimens of this midge which I have found myself have been taken on the flowers of umbellifers (*Angelica* and *Heracleum*), often in company with great numbers of some other species of *Atrichopogon*.

#### VI. A SPECIES ATTACKING A PHASMID

Williston (1908) mentions a minute fly which was found in the West Indies 'closely applied to and apparently sucking the juices from the antennae of a Phasmid.' He considered the specimen to represent a new genus of *Simuliidae*, but the figures which he gives indicate rather a Ceratopogonine midge. The available evidence is insufficient to place this species generically, though if Williston's figure of the wing is accurate, it would not seem to fit very well into any known genus. The specimen is not among the West Indian collections in the British Museum which were studied by Williston, and I am informed that it cannot be traced in those parts of his collection which are now in Washington and New York.

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# ON SOME STRONGYLID LARVAE IN THE HORSE, ESPECIALLY THOSE OF *CYLICOSTOMUM*

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The adult stage of species of *Cylicostomum* found in the large intestine of the horse has been extensively studied by Looss and, more recently, by Boulenger, and Yorke and Macfie (cf. Ihle, 1922), but very little is known as yet of the development of these species in the body of the horse.

The larvae of *Cylicostomum* are to be found in large numbers in the mucosa of the caecum and colon of the horse. They were first found by Dick (1836) and described by Knox (1836). By Diesing (1851, Vol. II, p. 332) they were mentioned under doubtful species as *Nematoideum equi caballi*. T. Spencer Cobbold (1874, p. 85) describes and figures *Cylicostomum* larvae as adult Nematodes under the name *Trichonema arcuata*, but the next year (1875, p. 241) he states that *Trichonema* is only the larval form of *Cylicostomum* ('*Strongylus tetracanthus*').

Short descriptions and sketches of the larvae, encysted in the mucosa of the large intestine, are also to be found in Leuckart (1876, p. 445), Cobbold (1886, p. 288) and Giles (1892, p. 15, Pl. III, figs. 16, 18). The last-mentioned author thought he had found a free-living *Rhabditis* generation of *Cylicostomum*. This mistake, which has been made repeatedly and also recently, when the development of different Nematodes was traced, is due to the fact that the cultures of larvae were infected with free-living Nematodes.

An investigation of Cuillé, Marotel and Roquet (1913), dealing with our subject, is of more importance than the older publications above mentioned. These authors distinguished three types of larvae, living in the mucosa of the large intestine of the horse and considered as belonging to *Cylicostomum*:—(1) 'La larve oesophagostomiforme,' with mouth-capsule and dorsal tooth and having a length of 2 to 5 mm.; (2) 'la larve metastrongyliforme,' without mouth-capsule (length  $800\mu$  to 2 mm.); and (3) 'embryons,' without recognisable internal structure (length  $300\mu$  to  $800\mu$ ). They showed that the 'larve oesophagostomiforme' passes over into the juvenile *Cylicostomum* by a moult.

Recently a part of the development of *Cylicostomum insigne* was shortly described by Boulenger (1921), who figures small larvae (6 to 7 mm. in length) and large larvae (up to 11 mm. in length), both agreeing with the 'larve oesophagostomiforme' of the French authors. In the larger larvae the adult mouth-capsule makes its appearance, which represents the preparation for the last ecdysis.

We ourselves have examined a large number of larvae, partly collected by the Commission appointed to inquire into Sclerostomiasis in Holland, and partly by ourselves in the horses dissected in the Anatomical Institute of the Veterinary College at Utrecht. All the larvae were found in the mucosa of the large intestine, though a small number were met with free in the lumen of the intestine.

The larvae examined by us can be divided into different types, to be described in subsequent pages. They belong for the greater part to *Cylicostomum*, a few perhaps to *Triodontophorus*; others were not identified. In addition we found a few very small larvae without recognisable internal structure. They agree with the so-called 'embryons' of Cuillé, Marotel and Roquet (1913, p. 8 of reprint), and were only obtained by us in a few cases by scratching the mucosa of the large intestine. We have not yet studied these forms in detail, but we do not think that these small worms (according to the French authors measuring  $300\mu$  to  $800\mu$  in length) must be considered to belong to the genus *Cylicostomum*, because it follows from the investigations of A. Albrecht (1909) and of De Blicck and Baudet (not yet published) that the larvae of *Cylicostomum* infecting the horse are much more differentiated.



## CYLICOSTOMUM LARVAE

All *Cylicostomum* larvae, found by us in the mucosa or in the lumen of the intestine, show a cup-shaped larval mouth-capsule, which has been already described by Cobbold (1874, p. 86). In agreement with this author (1886, p. 288) we will call this larval stage *Trichonema* stage. The name 'larve oesophagostomiforme' must be rejected, as these larvae do not in any particular agree with *Oesophagostomum*.

The cuticle is ringed. The cuticle surrounding the circular mouth-opening may also be called mouth-collar here; this larval mouth-collar, however, is much less developed than the adult one. The mouth-opening is generally surrounded by six papillae. External and internal leaf-crown are absent. The mouth-capsule is either sharply marked off from the mouth-collar or passes gradually over into it. In the middle the mouth-capsule is mostly wider, and possesses a thicker wall than posteriorly and anteriorly. Especially near the mouth-opening, the wall is very thin. The anterior part of the mouth-capsule is mostly provided exteriorly with a collar, often strongly developed, and which we will call mouth-capsule collar. It is divided into six lobes, which have a crescent shape and are almost perpendicular to the outer surface of the mouth-capsule. Between every two lobes a head-papilla is to be found.

An oesophageal funnel, in which the three sectors of the oesophagus continue, is present. The dorsal sector is always provided with a tooth, more or less protruding into the lumen of the mouth-capsule. The cuticular lining of the anterior margin of the oesophageal funnel shows a circular thickening, adjacent to the mouth-capsule. We will call this thickening the funnel-ring; it is directed to the exterior.

The oesophagus is cylindrical in shape and somewhat swollen posteriorly. Where the oesophagus passes over into the mesenteron three valves protrude into the lumen of the intestine. A nerve-ring, surrounding about the middle of the oesophagus, is present.

The mesenteron is composed for the greater part of a dorsal and a ventral row of alternating, polynuclear cells, which are mostly pigmented. In the anterior part of the mesenteron the cells are always much flatter than in the posterior part. In the anterior part

the cell-limits run transversely or directed obliquely to the front; so that the lateral parts of the cell-limits are situated more anteriorly than the dorsal and ventral parts.

The very short rectum opens into the exterior through the anus, situated at a small distance from the sharp posterior extremity of the body.

Sometimes, but not always, the larvae living in the mucosa are red in colour. It appears that in the few cases examined by us the whole body, the pigmented intestine excepted, is red. When such a larva is pricked, a red fluid is emitted. The juvenile specimens, living in the lumen of the colon and caecum, may also show this colour, but, as in the case of the larva, the intestine was not red in the specimens examined by us. Prof. B. Sjollem and Miss J. E. van der Zande were so kind as to analyse microchemically and spectroscopically a few juvenile specimens of *Cylicostomum insigne* for us. The fluid appeared to be due to oxyhaemoglobin.

We assume that the larvae living in the mucosa feed on blood at least during a part of their life. Boulenger (1921, p. 324) found these larvae in cysts, filled with blood; this is not always the case, however. As mentioned above, the red colour was not observed in the cells of the intestine of the larvae. We suppose that the larvae had fed on blood in an earlier period; consequently the red colour must have disappeared already from the intestinal wall, but not yet from the rest of the body. Further, we are of opinion that the red colour of the adult worm is the consequence of the larvae having fed on blood, for the adult *Cylicostomum* feeds on the contents of the large intestine of the host and not on blood. After dissection these worms are never found attached to the mucosa of the host's intestine.

The larvae, which we consider to belong to the genus *Cylicostomum*, can be divided into two types, to be described below. Not much importance must be attached to the dimensions indicated, as larvae of numerous species are brought together which when adult differ strongly in size. We cannot state to which species of *Cylicostomum* these different types belong, because we have not at our disposal a large enough number of moulting specimens.

*Cylicostomum* Larva. Type A (fig. 1).

To this type the smallest larvae of the genus *Cylicostomum* are considered to belong, having a length of 3 to 4.5 mm. and a maximum thickness of  $110\mu$  to  $200\mu$ . The mouth-margin is smooth. Around the mouth-opening the cuticle is thick. Head-papillae could not be observed. A mouth-capsule collar was not found by us. The length of the mouth-capsule, including the mouth-collar, varies from  $20\mu$  to  $28\mu$ .

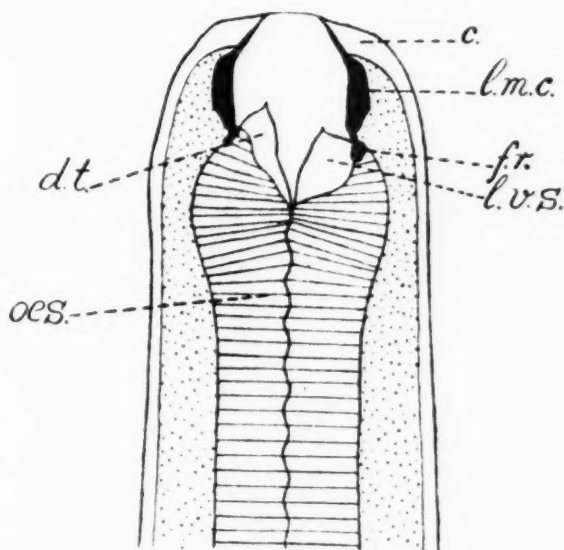


FIG. 1. Anterior extremity of a *Cylicostomum*-larva, type A, seen from right side.  $\times 540$  ( $\times \frac{3}{4}$ ). *d.t.*—Dorsal tooth; *oes.*—Oesophagus; *c.*—Cuticle; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—funnel-rings; *l.v.s.*—Latero-ventral sector of the oesophageal funnel.

In this type the oesophageal funnel also possesses three sectors. The dorsal sector always possesses a tooth, varying in size; the two latero-ventral sectors are rounded or bear an inconspicuous tooth, which never protrudes as far into the lumen of the oral capsule as the dorsal, large tooth. The length of the oesophagus varies from  $250\mu$  to  $350\mu$ . The distance from the anus to the posterior extremity of the body is  $80\mu$  to  $130\mu$ .

This type is very common.

*Cylicostomum* Larva. Type B (fig. 2).

Another type (B) is also of frequent occurrence. It differs from Type A in being of a larger size and in possessing a mouth-capsule collar. The length is 7.5 to 12.5 mm., the maximum thickness

420 $\mu$  to 580 $\mu$ . Six head-papillae are present, agreeing as to arrangement with those of the adult specimens; so there are two lateral and four sub-median papillae. The oral margin is mostly somewhat incised near the six papillae. Length of the mouth-capsule, including the mouth-collar 55 $\mu$  to 65 $\mu$ . At one-third of the length of the mouth-capsule from the posterior margin the wall of the oral capsule is thickest. The wall of the mouth-capsule becomes thinner anteriorly and passes gradually over into the mouth-collar. The mouth-capsule collar is very well developed and situated immediately under the cuticle of the anterior part of the body. Here the cuticle is somewhat thickened.

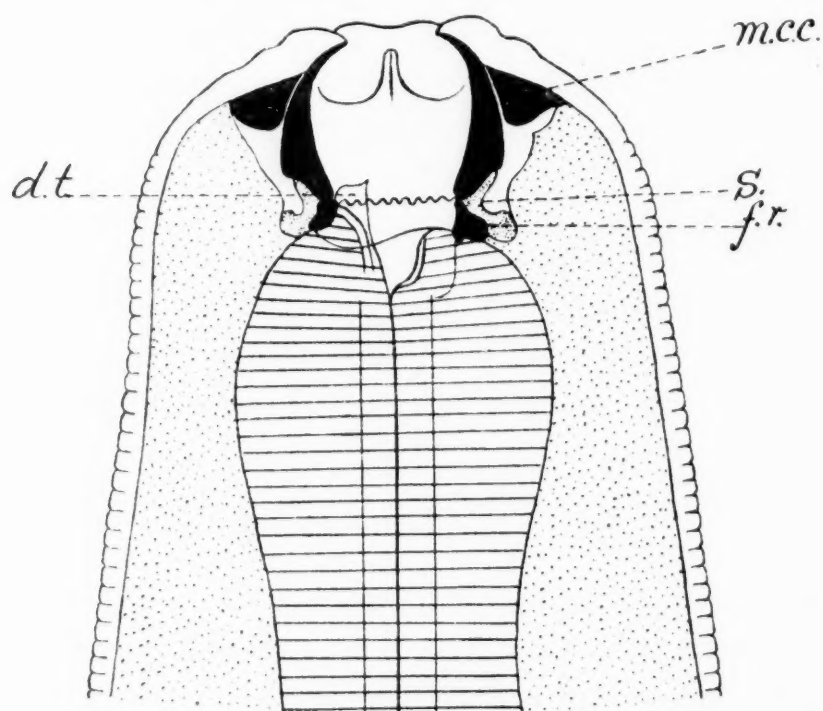


FIG. 2. *Cylicostomum*-larva, type B, seen from right side.  $\times 435 (\times \frac{2}{3})$ . *d.t.*—Dorsal tooth; *m.c.c.*—Mouth-capsule collar; *s.*—Septum; *f.r.*—Funnel-ring.

The oesophageal funnel is conspicuous and bears a cuticular lining of variable thickness. The funnel-ring is well developed. In some cases the border between mouth-capsule and funnel-ring is irregular or undulating. Sometimes the funnel-ring possesses a circular groove at its outer surface, so that in optical section it appears as a double ring. In this type, too, the dorsal sector of the oesophagus is continued as a tooth, protruding into the lumen of the oral capsule; this tooth is relatively not so large as in Type A.



The latero-ventral sectors are truncated anteriorly, or become lower and lower, to end at the funnel-ring. At the bottom of the grooves by which the sectors are separated the cuticle is thickened, just as in the adult worm. The oesophagus measures  $550\mu$  to  $650\mu$  in length; the distance from the anus to the extremity of the body is  $190\mu$  to  $220\mu$ .

We consider that the larvae belonging to Type B represent a more developed stage of Type A, because we have found several larvae with the rudiments of the mouth-capsule collar (fig. 3); these

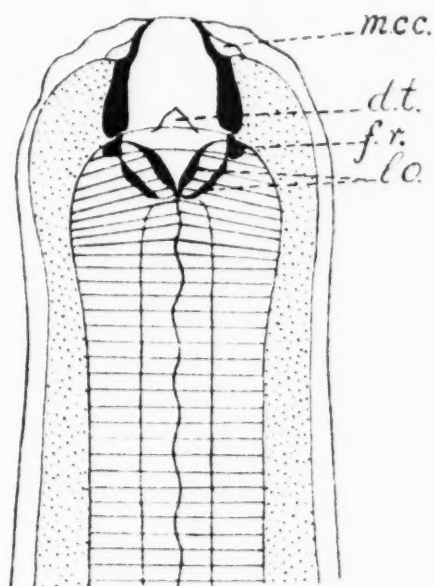


FIG. 3. *Cylicostomum*-larva, intermediate between types A and B, seen from dorsal side.  $\times 540$  ( $\times \frac{1}{4}$ ). m.c.c.—Mouth-capsule collar; d.t.—Dorsal tooth; f.r.—Funnel-ring; l.o.—Lining of the oesophageal funnel.

larvae are of a size intermediate between those of Types A and B. Length 5 mm. to 6.5 mm., maximum thickness  $350\mu$ ; length of the mouth-capsule, including the mouth-collar,  $42\mu$ ; oesophagus  $540\mu$  long.

#### *The last ecdysis* (figs. 4, 5).

The *Trichonema* stage passes over into the juvenile worm, living in the lumen of the large intestine, by a moult. In agreement with the development of other Nematodes, we assume that this moult is the fourth and last. The ecdysis itself takes place in the intestinal lumen.

The moult begins with the formation of a cavity around the larval mouth-capsule (fig. 2). We consider that one continuous cavity is



present from the beginning. Boulenger (1921, p. 325) mentions a series of cavities. However, according to Looss (1897, p. 925), two cavities (a dorsal and a ventral one) are formed in the larva of the fourth stage of *Ancylostoma*. Later on these cavities unite to form a circular lumen.

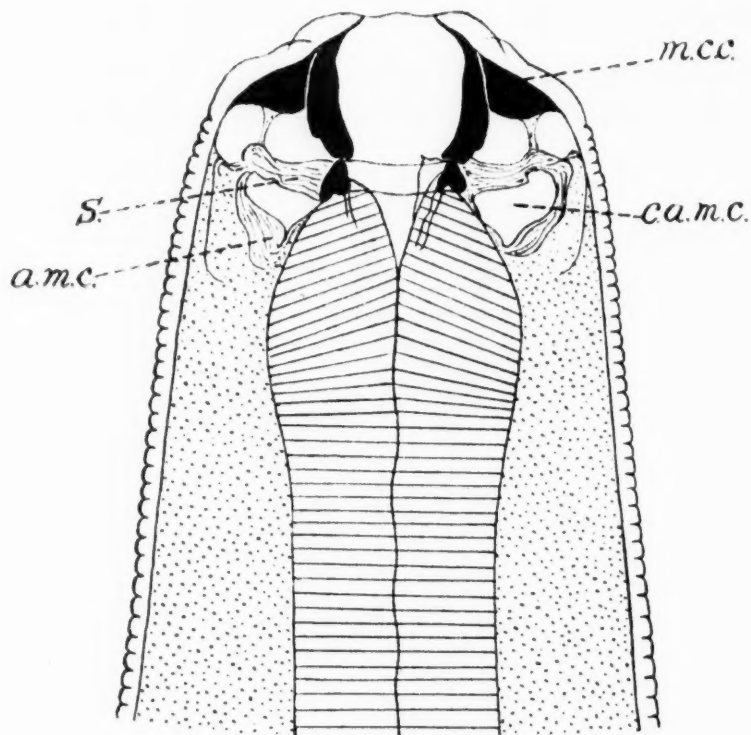


FIG. 4. *Cylicostomum*-larva, type B, seen from left side, with rudiment of the cavity of the adult mouth-capsule.  $\times 290 (\times \frac{3}{4})$ . s.—Septum; a.m.c.—Wall of the adult mouth-capsule; m.c.c.—Mouth-capsule collar; c.a.m.c.—Cavity of the adult mouth-capsule.

When the cavity makes its appearance, it is narrow at the front and a little wider backwards. Later on the anterior part of this cavity extends to the mouth-capsule collar. In the posterior part, which extends almost to the oesophagus, we see a granular substance, which seems to form a thin layer (fig. 2, s.) about at the level of the posterior margin of the mouth-capsule. This layer corresponds with the definitive anterior side of the mouth-capsule of the adult worm. This septum (fig. 4, s.) gradually becomes thicker, possibly formed by the granular substance mentioned above, while the cavity lying behind this septum, and in the beginning filled up with this substance, becomes empty and extends simultaneously backwards. This cavity, situated behind the septum, is the lumen of the adult mouth-capsule. At the periphery of the septum the definitive

mouth-collar and the definitive head-papillae develop (fig. 5). The cavity mentioned gradually widens and peripherally begins to form the wall of the adult oral capsule. Now this circular cavity surrounds the anterior part of the oesophagus (fig. 4).

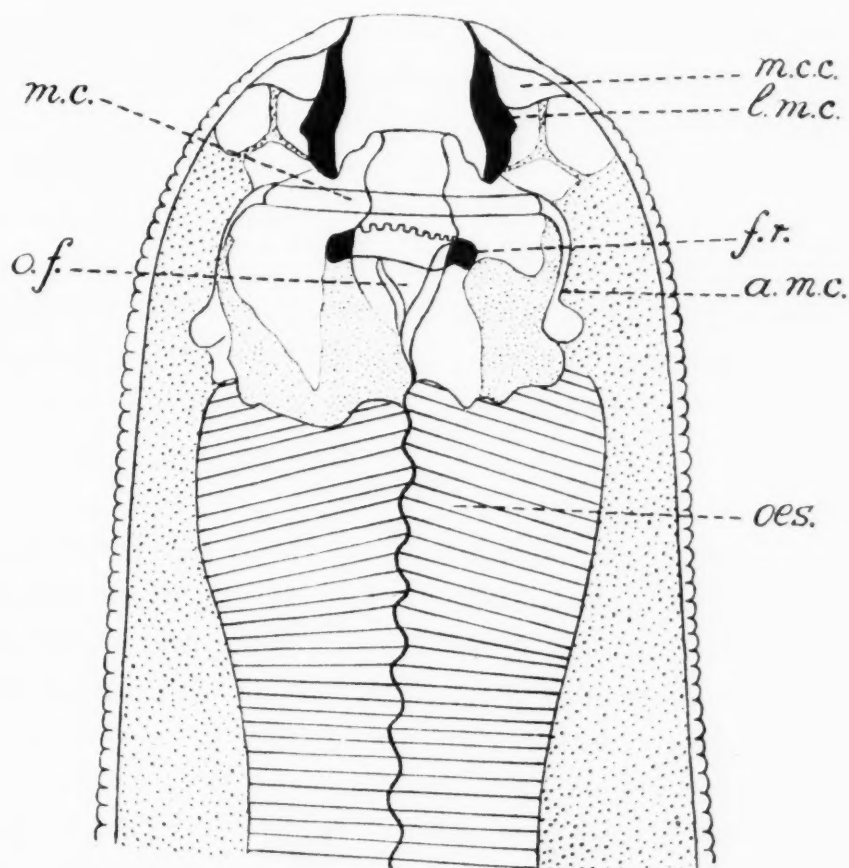


FIG. 5. *Cylicostomum*-larva, type B, moulting.  $\times 290 (\times \frac{3}{4})$ . *m.c.*—Mouth-collar; *o.f.*—Larval oesophageal funnel; *m.c.c.*—Mouth-capsule collar; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—funnel-ring; *a.m.c.*—Wall of the adult mouth-capsule; *oes.*—oesophagus.

Meanwhile the cuticle of the adult worm is formed below the provisional one. Before the ecdysis proper the oesophagus loosens itself from the cuticular lining of its funnel (fig. 5). In earlier stages the oesophagus tapers to the anterior extremity and ends at the funnel-ring (fig. 4). But at this stage it becomes truncated in front. Now the lumen of the mouth-capsule is situated before the oesophagus, whereas the anterior part of the latter was formerly surrounded by the adult mouth-capsule. Simultaneously the mouth-capsule and the funnel-ring, which remains connected with the cuticular lining of the provisional oesophageal funnel, separate. The posterior margin of the mouth-capsule and the anterior margin

of the funnel-ring remain connected by a thin membrane, of which the origin is difficult to trace. Boulenger (1921, fig. 5 *b*) also figures it, without describing it. In the moulting specimen sketched by Cuillé, Marotel and Roquet (1913, fig. 17, 7), in which the adult mouth-capsule and mouth-collar have developed completely, the funnel-ring and the provisional mouth-capsule are still connected. In the specimen sketched by us the external leaf-crown is already visible, but not indicated in the figure.

#### OTHER LARVAE

Besides the larvae Types A and B (*Cylicostomum*), we also found some other types, which we were unable to identify. Short descriptions, dealing only with the differences between these types and the larvae of *Cylicostomum*, will now be given.

#### LARVA. Type C (fig. 6).

This type and the following Type D do not differ essentially from the larvae of *Cylicostomum*. Type C was found only once in the mucosa of the large intestine; but three specimens could be investigated. The length is 4.5 to 6.6 mm., the maximum thickness

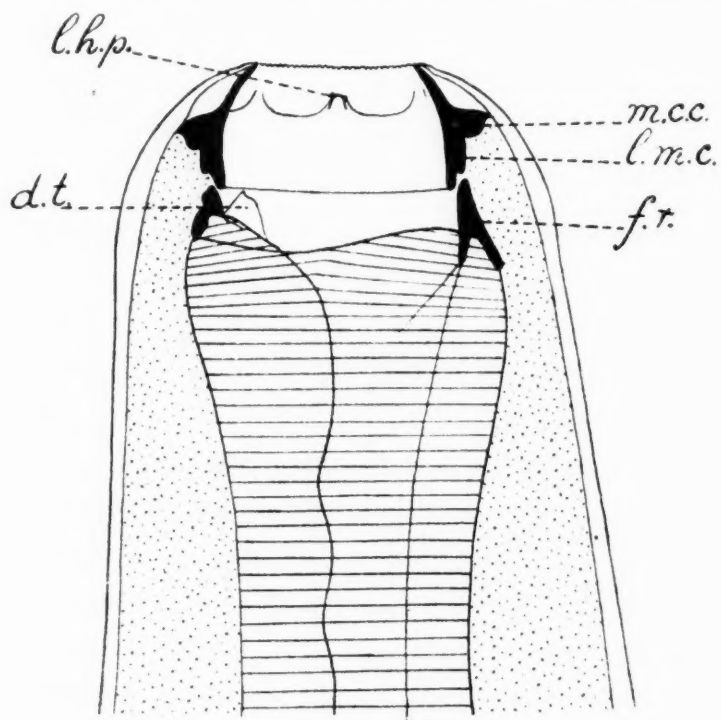


FIG. 6. Larva, type C, seen from right side.  $\times 310$  ( $\times \frac{3}{4}$ ). *l.h.p.*—Lateral head-papillae; *d.t.*—Dorsal tooth; *m.c.c.*—Mouth-capsule collar; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—Funnel-ring.

225 $\mu$  to 380 $\mu$ . The mouth-opening is circular, its margin is delicately denticulated. A mouth-collar is present, of which the side directed to the body-axis possesses a layer passing over into the anterior margin of the mouth-capsule. (In the figure the limit between mouth-collar and mouth-capsule is not indicated.) The head-papillae are present. The oral capsule is short and very wide; it is 52 $\mu$  to 65 $\mu$  in length, the mouth-collar included. The mouth-capsule collar is well developed and implanted in the anterior half of the mouth-capsule.

The oesophageal funnel is wide and bears a dorsal tooth. The latero-ventral sectors are smooth. The funnel-ring is very long at the dorsal side, shorter, however, than at the ventral side, where its length is equal to that of the mouth-capsule. In optical section this ventral part especially has the shape of an inverted Y, which encloses a part of the musculature of the oesophagus. The latter is short and thick, 435 $\mu$  to 550 $\mu$  in length, consequently measuring one-tenth to one-twelfth of the total body-length.

The posterior extremity is rounded. In one of the specimens examined, the part of the body situated behind the anus becomes gradually thinner; the distance from the anus to the posterior extremity is considerable (380 $\mu$ ) here. In two other specimens the thickness of this part diminishes suddenly at some distance behind the anus; consequently the body ends in an almost cylindrical point. In these cases the distance from the anus to the posterior extremity is 155 $\mu$  to 180 $\mu$ . Possibly these are sexual differences.

#### LARVA, Type D (fig. 7).

We found this type five times (only a few specimens) in the mucosa of the large intestine. We do not know whether this type and also the former (Type C) belong to *Cylicostomum*. Length 3.5 to 5.1 mm., maximum thickness 120 $\mu$  to 190 $\mu$ . The head-papillae are present. The cuticle is swollen around the mouth-opening; here the part of the cuticle directed to the body-axis possesses a particular layer, which passes over into the mouth-capsule. The latter is 25 $\mu$  to 27 $\mu$  long, the cuticle surrounding the mouth-opening included. Posteriorly its wall increases in thickness. A slightly developed mouth-capsule collar is present, lying immediately against the cuticle.



The dorsal sector of the oesophageal funnel bears a large tooth, protruding far into the lumen of the mouth-capsule. At the dorsal side its anterior margin possesses a small point and at the ventral side a large one. Each of the latero-ventral sectors bears a small tooth with one point. Moreover, the well-developed funnel-ring

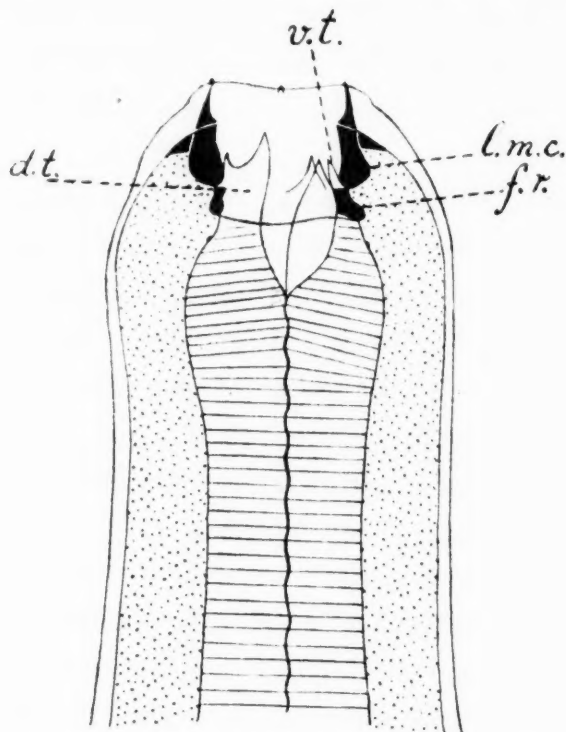


FIG. 7. Larva, type D, seen from right side.  $\times 540$  ( $\times \frac{3}{4}$ ). *d.t.*—Dorsal tooth; *v.t.*—Ventral tooth; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—Funnel-ring.

possesses medio-ventrally a pointed tooth, being directed anteriorly (fig. 7, *v.t.*). Peripherally the funnel-ring does not protrude markedly. The oesophagus is long ( $400\mu$  to  $435\mu$ ), being one-ninth to one-twelfth part of the body-length. The mesenteron agrees with that of *Cylicostomum* larvae. The anus is situated  $105\mu$  to  $115\mu$  from the posterior extremity of the body.

#### LARVA. Type E (fig. 8).

We found this type only once in four specimens in the lumen of the large intestine. We consider that these larvae belong to *Triodontophorus*. It is, however, very remarkable that we found this type only once in our comprehensive material, as two *Triodontophorus* species are common in Holland, and sometimes inhabit one host in large quantities.



The length of these four larvae is 7.6 to 8.5 mm., the maximum thickness  $310\mu$  to  $365\mu$ . The mouth-opening is circular and surrounded by a thin mouth-collar, finely and longitudinally striated, and resembling an extremely little developed external leaf-crown. The six head-papillae are distinctly visible. The length of the mouth-capsule (including the mouth-collar) is  $65\mu$  to  $82\mu$ . The mouth-capsule is wide, cup- or barrel-shaped, and sharply marked

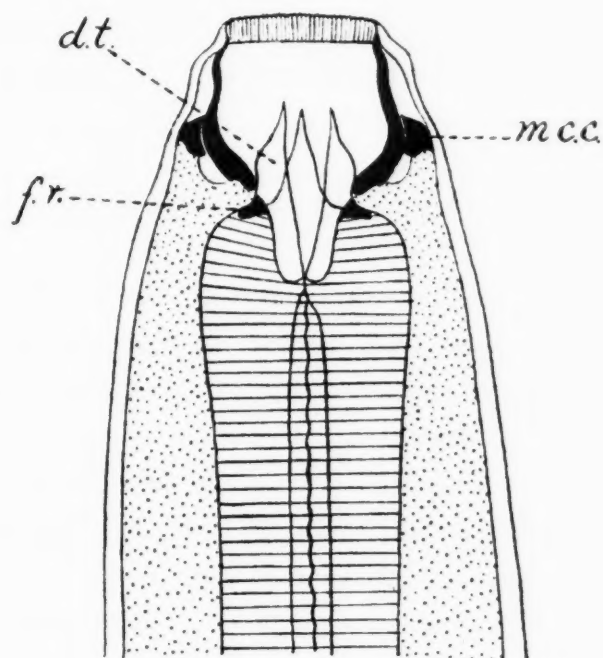


FIG. 8. Larva, type E, seen from right side.  $\times 335$ . ( $\times \frac{3}{4}$ ). *d.t.*—Dorsal tooth; *f.r.*—Funnel-ring; *m.c.c.*—Mouth-capsule collar.

off from the mouth-collar. In some of the specimens it lies immediately against the cuticle. (The space between cuticle and mouth-capsule in the specimen figured is possibly due to the preservation.) The mouth-capsule collar reaches very far posteriorly beyond the equator of the mouth-capsule. Around the posterior part of the latter a circular cavity is already visible: the rudiments of the lumen of the adult mouth-capsule.

The oesophagus is long ( $750\mu$  to  $820\mu$ ), being about one-tenth of the body-length. The oesophageal funnel is well developed. It bears three large, pointed teeth, which protrude far into the lumen of the mouth-capsule. The dorsal tooth is a little larger than both the latero-ventral teeth. A funnel-ring is present. The distances from the anus to the rounded posterior extremity of the body is in

two specimens respectively  $100\mu$  and  $110\mu$ , in both other specimens respectively  $220\mu$  and  $240\mu$ ; possibly these are sexual differences.

We suppose that these larvae belong to *Triodontophorus*, because the oesophageal funnel bears three large teeth protruding into the lumen of the mouth-capsule, this characteristic being present among the adult Strongylids of the horse, in *Triodontophorus* only. Moreover, the great length of the oesophagus of this type agrees with the long oesophagus in *T. intermedius* and *T. brevicauda*. For the rest, no other larva was present in our material which could be considered to belong to *Triodontophorus* on better grounds.

LARVA. Type F (fig. 9).

Besides the larvae described above, which all possess a well-developed mouth-capsule, we found in the mucosa of the large intestine of the horse in one case one larva without mouth-capsule.

Length  $3.7$  mm., maximum thickness  $190\mu$ . In front of the oesophagus, having a length of  $350\mu$ , is a tube-shaped mouth-cavity, projecting slightly above the level of the anterior extremity of the body and being spherically swollen posteriorly.

We cannot decide whether this larva is identical with the 'larve metastrongyliforme' of Cuillé, Marotel and Roquet, which, however, is shorter (length  $800\mu$  to  $2$  mm.) than the specimen found by us.

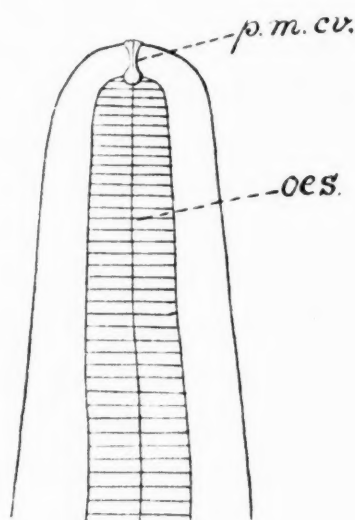


FIG. 9. Larva, type F.  $\times 340$  ( $\times \frac{3}{4}$ ). *p.m.cv.*—Provisional Mouth-cavity; *oes.*—Oesophagus.

Possibly both the larvae observed by the French authors and the specimen found by us are identical with a larva encountered by Leuckart (1876, p. 446) in the mucosa, being 1 mm. long and differing 'durch die Abwesenheit des Mundbechers, dessen Stelle durch einen schlanken und dünnhäutigen Chitincylinder vertreten war, wie bei den ersten parasitischen Jugendzuständen des *Dochmius trigonocephalus*. Die Umwandlung in die Form mit Mundbecher geschieht durch eine Häutung, die schon bei Exemplaren von 1.5 mm. vollendet ist.' If the supposition above made proves to be correct, Type F represents the third larval stage of *Cylicostomum*, though the differences between the encysted larvae (larvae of the third stage, enclosed in the cuticle of the second stage) and Type F are conspicuous.

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# AVIAN CESTODES FROM NEW GUINEA

## II. CESTODES FROM CASUARIFORMES

BY

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*(Received for publication 5 December, 1922)*

As it has been pointed out in the first part of this paper (Kotlán, 1921), the worms described in both the former and present notes, as well as those which will be described subsequently, belong to a rather large collection of parasites which were partly sent, partly brought back, by the Hungarian naturalist, Lewis Biró, from the formerly German New Guinea, during the years 1897-1899.

The intestinal parasites of birds belonging to the Casuariformes are represented in this collection by numerous Cestodes from *Casuarus picticollis*, Sclat. A hasty examination of these worms showed that they all belong to the family of Davaineidae. On account of external features two species could be distinguished, a larger and a smaller one, both belonging to the genus *Davainea*, R. Bl. (s. l.). Until now only one representative of this genus was known from Casuariformes,\* viz., *D. australis* (Krabbe, 1869), from *Dromaeus novae hollandiae*. This species, however, is easily separated from the two above apparently undescribed forms.

### *DAVAINEA* (s. l.) *CASUARII*, sp. n.

Host: *Casuarus picticollis*, Sclat.

Locality: Erima and Sattelberg.

The majority of the worms collected from this host—about two hundred more or less developed specimens—belong to this new

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\* Meggitt (1921) quotes in his key to the species of *Davainea* two '*Davainea*, sp. nov., Vevers, 1920' from *Casuarus uniappendiculatus* Blyth. I have been unable to obtain Vevers' paper (*Proc. Zool. Soc.*, 1920.).



species. The strobilae, coming from the two above-mentioned localities, exhibit in their external appearance a well marked difference, for those from Sattelberg are much more contracted and have a shorter, almost cylindrical body, while those from Erima are more stretched and thus longer in size. The largest specimens measure 34 cm., the greatest width (3 mm.) occurs in the posterior part of the strobila. The worms, which are in an expanded condition, bear a well marked scolex, which is short and approximately square, its diameter being 1 to 1.2 mm., while in contracted worms it is not clearly marked off from the strobila. It also happens sometimes that the anterior end of the strobila bears by means of unequal contraction a pseudoscolex-like thickening of 3 to 5 mm. length, with the true scolex at the end, as is shown in fig. 1. The



FIG. 1. *Davainea casuarii*, n.sp. Showing the extremely contracted anterior end of the body with the scolex.  $\times 17$ .

rather muscular rostellum measures 0.5 mm. in breadth, and is armed with two hundred and fifty very large hammer-shaped hooks, which are arranged in two rows. The hooks of the anterior row are  $48\mu$  to  $54\mu$ , those of the posterior row  $40\mu$  to  $46\mu$  in length. As far as I am aware, there is only *Houttuynia struthionis* (Houtt.) which has larger hooks, all the other members of the genus *Davainea* (s.l.) bearing smaller ones. In the following table are enumerated some

*Davainea* species the rostellar-hooks of which are comparatively the largest ones and measure over  $20\mu$  in length :—

Species	Host	No.	Length
		of rostellar-hooks	
<i>Houttuynia struthionis</i> * (Houtt.) ... ..	<i>Struthio molybdophanus camelus</i> ... ..	1641	$65-80\mu^2$
<i>Davainea</i> (s.l.) <i>casuarii</i> , sp.n. ... ..	<i>Casuaris picticollis</i> ... ..	250	$40-54\mu$
„ <i>appendiculata</i> , Fuhrm. ... ..	Unknown ... ..	130	$36-43\mu$
„ <i>infrequens</i> , sp.n. ... ..	<i>Casuaris picticollis</i> ... ..	260	$21-34\mu$
„ <i>fuhrmanni</i> , Southwell ... ..	<i>Crocopus phoenicopterus</i> ... ..	110	$25-30\mu$
<i>Raillietina</i> ( <i>Ransomia</i> ) <i>undulata</i> , Fuhrm. ...	<i>Corythaeola cristata</i> ... ..	150-200	$25-28\mu$
„ „ <i>campanulata</i> , Fuhrm. ... ..	<i>Perdix</i> sp. ... ..	40-42	$27\mu$
„ „ <i>vaganda</i> (Baylis) ... ..	<i>Haliaetus vocifer</i> ... ..	numerous	$25\mu$
„ ( <i>Paroniella</i> ) <i>paradisea</i> , Fuhrm. ... ..	<i>Manucodia chalybeata</i> ... ..	about 100 <sup>3</sup> about 200 <sup>4</sup>	$23\mu$ $22\mu$
„ ( <i>Skrjabinia</i> ) <i>oligacantha</i> , Fuhrm. ... ..	<i>Tynamus</i> , sp. <i>Rhynchotus rufescens</i> ... ..	34	$21-23\mu$
<i>Davainea</i> (s.l.) <i>conopophila</i> , Johnston ...	?	?	23

\* According to Meggitt (1921) *T. struthionis*, as described by different authors, contains more than one species, and reserving the name *Davainea struthionis* for the form firstly mentioned (without proper description) by Houttuyn and described 1885 by Parona, he separates from this latter the following species: *D. linstowi* Meggitt (1921) (= *T. struthionis* of v. Linstow (1893) and Hungerbühler (1910)) and *D. beddardi* Meggitt (1921) (= *D. struthionis* of Zilluf (1912)). The size of the rostellar-hooks is stated to be different in all the three species.

1. According to v. Linstow (1893).
2. According to Fuhrmann (1920).
3. According to Fuhrmann (1909).
4. According to Skrjabin (1914).

The four suckers are rounded in size, and exhibit a well pronounced musculature; they measure 0.4 mm. across; their border is covered with very numerous small ( $10\mu$  to  $13\mu$ ) hooks, which are arranged in six to ten rows. A distinct neck occurs only in stretched specimens. The segments, in most of my specimens, are much broader than long. Gravid proglottides are, apart from extremely contracted specimens, almost square.

#### ANATOMY.

As has been mentioned above, the worms are in part greatly contracted, and their aspect is rather thick and compact. Such conditions are to be found usually in worms which possess a well

developed cortical parenchyma, subcuticular layer and cuticle. In *D. casuarii* especially the first is rather wide and exhibits a well marked longitudinal musculature. This latter consists of many more or less distinctly separated bundles of various size. The largest bundles are oval in shape, measuring about  $40\mu$  to  $54\mu$ . They are composed of thirty-five to fifty fibres of various thicknesses. Towards the subcuticular layer smaller bundles are scattered irregularly, consisting of fewer fibres, or even of but a single one. The

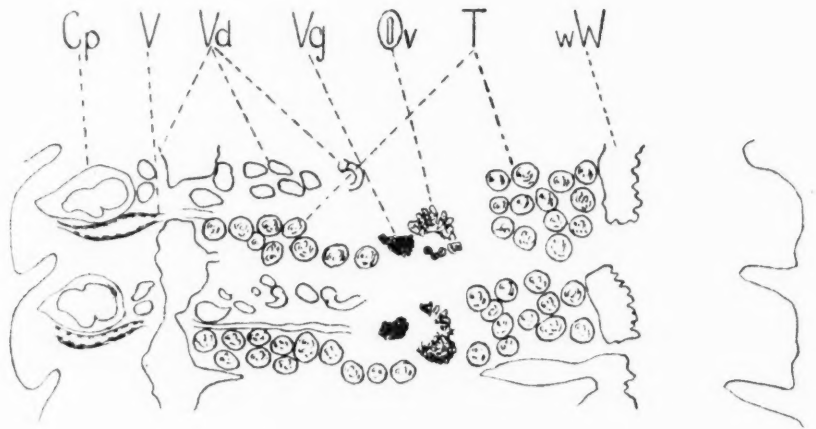


FIG. 2. *Davainea casuarii*, sp.n. Longitudinal section of two mature segments Cp.—cirrus pouch; Ov.—ovary; T.—testes; wW.—ventral excretory vessel; V.—vagina; Vd.—vas deferens; Vg.—vitelline gland.  $\times 34$ .

transversal musculature separates very distinctly the medullary parenchyma from the cortex. Fine dorso-ventral fibres are present in both parenchyma layers, being especially well marked at the level of the transverse excretory vessel. It is worthy of note that rather large calcareous bodies are scattered in the subcuticula as well as in both parenchyma layers; they are mostly oval in shape,  $10\mu$  to  $16\mu$  in size, and deeply staining with haematoxylin.

*Excretory system.* The excretory system consists in main part of a single pair of very large longitudinal vessels, which are connected at the posterior border of each proglottis by a large transverse canal. Although these longitudinal vessels run justly on the transverse axis of the proglottides, having a diameter nearly equal to the depth of the medullary parenchyma, there is no doubt that they represent the ventral pair of the longitudinal vessels, for in the anterior, mostly immature proglottides, I could undoubtedly distinguish within the two large vessels two narrow, somewhat

dorsally located vessels without transverse commissures. These dorsal vessels disappear apparently in the mature segments. The wall of the excretory vessels is bordered by very minute rounded cells, which seem to be parenchyma cells.

*Genital organs.* The openings of the genital ducts are unilateral, the porus genitalis being situated about the centre of the lateral border of the proglottides. A small atrium genitale is present.

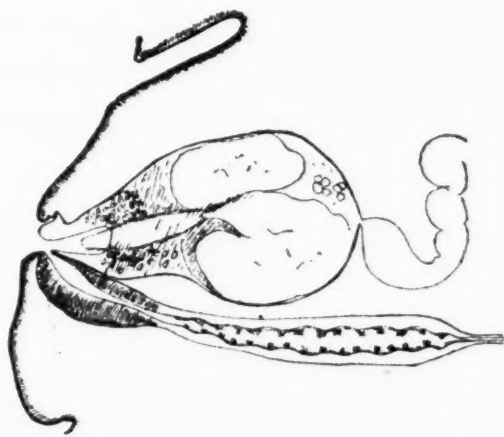


FIG. 3. *Davainea casuarii*, n.sp. Longitudinal section, showing the termination of sex ducts.  $\times 80$ .

*Male organs.* The testes are oval or spherical in shape and  $67\mu$  to  $81\mu$  in diameter. They occupy the whole free space of the medulla at the sides of the female glands. On account of the structure of the vas deferens and vagina, the testes are of course more numerous on the antiporal medulla-half. Their total number amounts to nearly fifty to sixty. In the younger, and also in mature proglottides, the testes exhibit very interesting stages in the development of the spermatozoa. These stages agree in many respects with those described and drawn by Moniez (1881). The vas deferens is a rather wide, coiled tube, the coils of which occupy dorso-ventrally nearly the whole space of the poral medulla-half, displacing ventrally the wide longitudinal excretory vessel just before entering the cirrus pouch. After entering the cirrus pouch, the vas deferens forms a rather large, coiled vesicula seminalis interna, which is usually filled with spermatozooids. The cirrus is short (0.1 mm.); it is surrounded within the cirrus pouch by a dense network of very small cells, representing, perhaps, prostate cells or merely parenchyma cells.

The thick-walled cirrus pouch is pyriform, and measures 0.25 mm. in length by 0.16 mm. in breadth; it does not extend beyond the longitudinal nerve-stem, and thus does not reach at all the longitudinal excretory vessel.

*Female organs.* The position of the vagina, i.e., of the poral portion of the vagina in proportion to the cirrus pouch, varies according to the state of contraction of the strobila. In stretched or normally contracted specimens it lies immediately behind the cirrus pouch; in extremely contracted worms, however, it is sometimes ventral, sometimes dorsal to the cirrus pouch. The poral third of the vagina, extending from the genital atrium just beyond the poral longitudinal excretory vessel, is rather wide, darkly

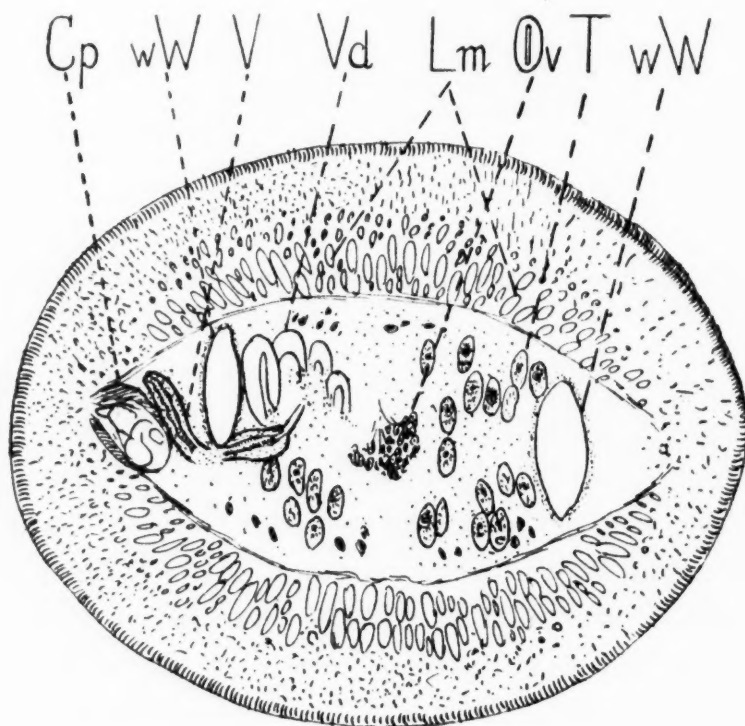


FIG. 4. *Davainea casuarii*, sp.n. Transverse section of a mature segment. Cp.—cirrus pouch; Lm.—longitudinal muscles; Ov.—ovary; T.—testes; wW.—ventral excretory vessel; V.—vagina; Vd.—vas deferens.  $\times 42$ .

staining because of its rather muscular wall and especially on account of the presence on its inner surface of very fine hairs. A similar structure of the vagina is found in other worms, particularly in some members of the family Davaineidae, in the Tetrabothriidae, and also in certain species of the genera *Trichocephaloides*,



*Monopylidium*, *Octopetalum*, etc. Just before the vagina opens into the atrium genitale it bears a distinctly marked sphincter. Within the poral longitudinal excretory vessel the vagina narrows suddenly for a short distance and becomes then nearly as wide again as the vas deferens, forming some coils before reaching the ovary; its course from the longitudinal excretory vessel to the ovary is chiefly ventral, although it passes to the dorsal side of the longitudinal excretory canal, as is usually the case. A distinct receptaculum seminis is absent. The ovary is small; it is situated in the middle of the proglottides lying in longitudinal sections somewhat nearer to the posterior border of the segments and consisting of fine lobes, which radiate in all directions from the oviduct. Its diameter amounts nearly to one-fourth of the breadth

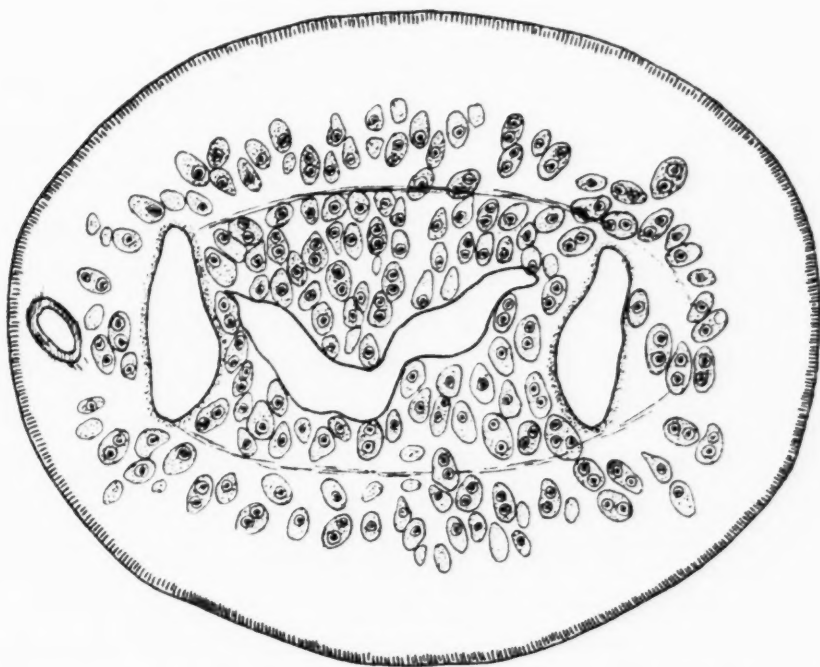


FIG. 5. *Davainea casuarum*, sp.n. Transverse section of a gravid segment.  $\times 34$ .

of the medullary parenchyma. Dorso-ventrally it occupies the entire depth of the medulla, being especially well developed on the antiporal side. The vitelline gland lies quite dorsally in the median part of the medulla, where it appears as a rounded compact organ, measuring about 0.01 by 0.06 mm. A small but distinct shell-gland lies on the main trunk of the oviductus. The uterus appears very early as a rounded sac, which is situated ventrally in the middle part of the medullary parenchyma. While enlarging it becomes apparently

divided into two oval parts, both becoming confluent soon after they reach a larger size. At this stage the eggs segregate into groups of three to four eggs, which latter eventually become egg-capsules; these are mostly oval or rounded in shape; they are bordered by one to two rows of rounded, larger cells, while the two to four eggs (in transverse sections there are visible mostly two, seldom three, eggs in a capsule) are embedded in dense parenchyma containing somewhat smaller cell-elements. The egg-capsules extend beyond the longitudinal excretory vessels, and thus fill the whole parenchyma. About one hundred and eighty capsules can be counted in a transverse section; they measure  $67\mu$  by  $108\mu$  in diameter.

#### *Systematic comparisons.*

As already mentioned above, there has been only one species of the genus *Davainea* hitherto known from birds belonging to the Casuariformes, viz., *Davainea* (s.l.) *australis* (Krabbe). *D. casuarii*, sp. n., differs from this cestode in many respects, but especially in the shape and size of the scolex and the rostellar-hooks. Comparing this new cestode with other members of the genus *Davainea* (s.l.), I find that it agrees in general with the type known in *Davainea*. There is, however, no doubt that it bears some characters which are to a certain degree rare or unusual in this genus; such features are the considerable size of the rostellar-hooks, the absence of one pair of excretory vessels and the well-developed longitudinal musculature. Owing to these peculiarities it seems that there exists a certain relationship between *D. casuarii* and the genus *Porogynia*, Railliet et Henry (= *Polycoelia*, Fuhrm.). The arrangement of the genital glands, however, which in our cestode is of the usual type of *Davainea*, does not allow it to be assigned to *Porogynia*, which latter, moreover, bears three rows of rostellar-hooks on the scolex. On the other hand, it is not possible to place this cestode into one of the genera recently established by Fuhrmann (1920), mainly because of the above-mentioned unusual features. Among all these new genera it is to the large genus *Raillietina*, Fuhrm., sub-genus *Ransomia*, Fuhrm., that our cestode should be assigned, if we do not consider the above-mentioned characters to

be of systematic value, warranting the creation of a new genus or sub-genus for it. For myself, I am inclined to believe that the establishment of a new sub-genus might be justified.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

*DAVAINEA* (s.l.) *INFREQUENS*, sp. n.

Host: *Casuarus picticollis*, Sclat.

Locality: Sattelberg.

Only a few specimens of this cestode were found in the same host as *D. casuarii*. The worms are much smaller and narrower than the former species. Unfortunately there were only incomplete individuals available. From two fragments, which belong apparently to one another, one can estimate the total length of the strobila at about 80 mm. by a greatest breadth of 1.2 mm. in the posterior third. The scolex is globular, measuring 0.5 mm. across. It exhibits a fairly well developed rostellum of 0.25 mm. in diameter, bearing a double row of typical hammer-shaped hooks. Their number is about two hundred and sixty. They measure in the anterior row  $27\mu$  to  $34\mu$ , in the posterior row  $21\mu$  to  $25\mu$  in length. The suckers are spherical, their diameter being 0.13 mm. They are bordered by four to six rows of hooks,  $10\mu$  to  $15\mu$  in length. There is a well marked neck measuring about 2 mm. in length. The proglottides are broader than long.

ANATOMY.

The internal anatomy of the worms exhibits the usual characters of the genus *Davainea*; the structures seen in transverse and longitudinal sections were much like those found, especially in some *Davainea* species of Psittaciformes.

*Excretory system.* There is only one pair of large longitudinal vessels, lying usually nearer to the ventral side.

*Musculature.* The longitudinal muscles are well developed; they are composed of an internal layer consisting of about sixty

large oval bundles, and by a distinctly separated external layer, which exhibits two rings of very small bundles consisting of at most two to three fibres. Similar arrangement of the longitudinal musculature occurs also in *D. spiralis*, Baczynska (1914).

Oval calcareous bodies are present, especially in the cortical parenchyma.

*Genital organs.* It seems that the openings of the sex-ducts are unilateral, lying on the left side; in one segment (of about twenty), however, I found the opening on the right. The thick-walled cirrus pouch is 0.18 to 0.2 mm. in length, 0.06 mm. in breadth; it extends to the poral longitudinal excretory vessel. The cirrus is short, rather thick at its anterior end and covered with many spine-like elements. It bears retractor muscles which radiate in all directions to the wall of the cirrus pouch. Within this organ there is an oval vesicula seminalis interna measuring 0.054 mm. in length. The vas deferens forms many large coils in its course towards the middle of the medulla. The testes are about nine to twelve in number, lying not only at both sides of the female glands, but also in the median line of the segments. They measure 0.05 mm. in diameter.

The vagina lies behind the cirrus pouch. Its structure is the same as, e.g., in *D. aruensis*, Fuhrm. (1911) or in *D. allomyodes*, Kotlán (1921). In the middle of the segments, shortly before reaching the ovary, it forms a small spindle-shaped receptaculum seminis. The bilobed ovary, when fully developed, is about 0.2 mm. in breadth; it lies in the middle of the segments. Behind the ovary is situated the compact vitelline gland, which is about 0.08 mm. broad.

Gravid proglottides are not available, and I am, therefore, unable to give a complete description of this worm. The above noted characters are, however, I believe, sufficient to distinguish this form from other members of the genus *Davainea*, of which the following must be considered mostly on account of the similar size of the rostellar-hooks:—*D. fuhrmanni*, Southwell (1922), *Raillietina* (*Ransomia*) *undulata*, Fuhrm. (1909), *R. (R.) campanulata*, Fuhrm. (1909), and *R. (R.) vaganda*, Baylis (1919).

The new worm in question seems in every way to be closely related to *D. fuhrmanni*. Comparing, however, the characteristic features of our worm with those of *D. fuhrmanni*, described in



detail by Southwell (1922), I conclude that there are some differences which do not permit the two species to be united. Such are:—

1. The smaller number of the rostellar-hooks in *D. fuhrmanni*.
2. Larger and, in some respects, better preserved material would perhaps show that the genital openings are irregularly alternate in *D. infrequens*.
3. It seems that the cirrus pouch in *D. infrequens* is longer and rather narrower in size.
4. There is no mention in the description of *D. fuhrmanni* of the distinct vesicula seminalis interna.
5. No mention is made of the presence in *D. fuhrmanni* of retractor muscles of the cirrus.
6. The prostate-cells surrounding the coils of the vas deferens are inconspicuous in *D. infrequens*.
7. Finally, it seems improbable that one and the same species of worm should be found in Columbiform and Casuariform birds.

The three other species mentioned above differ from *D. infrequens* in the number of the rostellar-hooks and in other anatomical characters.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

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## AVIAN CESTODES FROM NEW GUINEA

## III. CESTODES FROM GALLIFORMES

BY

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Only one representative of the bird-group Galliformes has been examined for parasites, viz., *Megapodius brunneiventris*, Mey. The tapeworms which were found in the gut of this bird belong to three distinct species of the genus *Dilepis*, Weinl; one of these is smaller and narrower than the two other species, and is, therefore, easy to separate from these latter. It requires, however, a careful examination to be able to distinguish the two other species, the scolex and strobila of which are quite similar to one another. All three species are, I believe, undescribed; the genus *Dilepis*, so far as I am aware, has not yet been recorded from Galliform birds.

*DILEPIS YORKEI*, sp. n.

Host: *Megapodius brunneiventris*, Mey.

Locality: Friedrich-Wilhelmshafen.

This is the smallest of the three species mentioned above; fully matured specimens measure 15 to 20 mm. in length. The scolex is

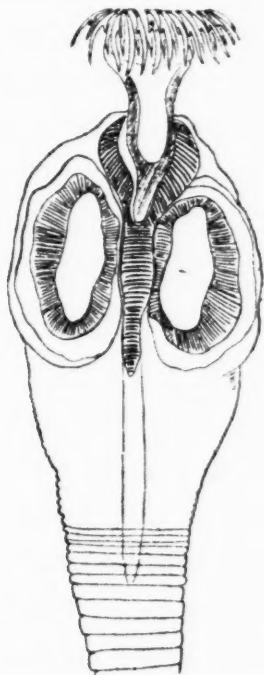


FIG. 1 *Dilepis yorkei*, sp.n. Scolex.  $\times 50$ .

very well developed, it is nearly as long (0.7 mm.) as broad (0.5 to 0.6 mm.). It bears a rather powerful rostellum of conical shape and of about 0.5 mm. in length. This rostellum, when retracted, is surrounded by a double muscular rostellar sac of about 0.7 mm. in length. On the anterior end of the rostellum there is a button-like thickening of nearly 0.17 mm. in diameter, bearing fifty to fifty-two large hooks, which are arranged in a double row.

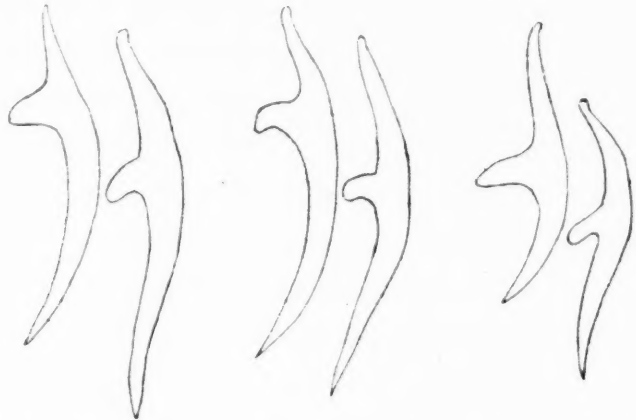


FIG. 2. Hooks from the rostellum. A.—*D. yorkei*; B.—*D. leptopallus*; C.—*D. borvathi*.  $\times 230$ .

They measure in the anterior row  $135\mu$ , in the posterior row  $148\mu$  to  $151\mu$ . The suckers are oval in shape and measure 0.42 to 0.44 by 0.25 to 0.30 mm. in diameter.

Behind the scolex there is a very short unsegmented portion, which is usually broader than the segments of the anterior half of the worm. The strobila of a fully developed specimen consists of about one hundred and twenty to one hundred and fifty segments; these are, as a rule, broader than long, except in macerated specimens. Mature segments are 0.2 to 0.4 mm. in breadth and 0.05 to 0.1 mm. in length. The greatest breadth (0.3 to 0.5 mm.) is attained in the last fourth of the strobila with gravid segments.

#### ANATOMY.

*Body-wall and parenchyma.* The cuticle, as in other similarly delicate cestodes, is rather thin and not at all compact. The subcuticular cells are fairly well developed and arranged into two or three rows. The body-parenchyma is of peculiar structure, consisting of a loosely arranged reticulum with rather poorly scattered cell-elements. Calcareous bodies were not found.

*Musculature.* The somewhat denser cortex is separated from the very loose medulla by the longitudinal muscles, which are arranged in two rings, each being composed of a row of small inconspicuous muscle-bundles. Inside of the interior row there is apparently a very poorly developed transverse musculature. Dorso-ventral muscle-fibres were not seen.

*Excretory system.* In the anterior two-thirds of the strobila there exist two longitudinal vessels on each side of the segments, of which the ventral is slightly larger than the dorsal. In segments in which the uterus reached a more considerable extent, only one pair of longitudinal vessels can be seen. Transverse commissures were not observed.

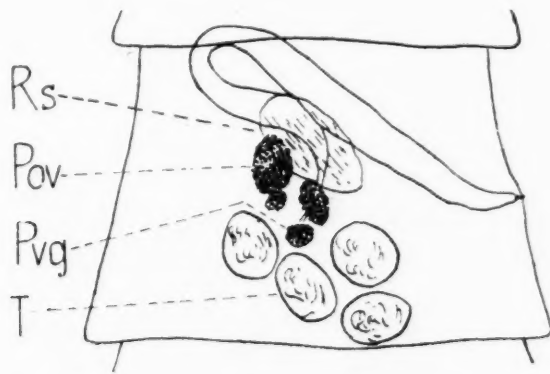


FIG. 3. *D. yorkei*, sp.n. Younger segment showing mature male organs and primordial female glands. *Pov.*—primordial ovary; *Pvg.*—primordial vitelline gland; *Rs.*—receptaculum seminis; *T.*—testes.  $\times 170$ .

*Genital organs.* The first indication of the sex-organs appears already in the first distinct segments. As in many other cestodes, the male organs are markedly more advanced in development than the female organs. The cirrus pouch attains its largest size by the twentieth segment; then follow the testes, which, however, disappear about the sixtieth to seventieth segment, while the female glands, which also appear rather early, reach full maturity after and about the eightieth segment. Here also the uterus appears, and grows rapidly to a considerable size.

The genital-openings are unilateral, being situated about the middle of the lateral border of the proglottides.

*Male organs.* The cirrus pouch, compared with the size of the proglottides, is a large tube of 0.18 to 0.2 mm. length and 0.021 to 0.027 mm. greatest breadth. Its position varies according to the

state of contraction of the worm. In somewhat longer segments it is directed obliquely to the anterior end of the segment. After narrowing for a short distance it is continued by a very wide vas deferens, which, forming one or two large coils, runs to the posterior half of the segment. The cirrus seems to be a fairly slender canal, which on its anterior end is apparently covered with minute spines.

There are only four testes in each segment, situated in the middle of the posterior third; they are  $37\mu$  by  $27\mu$  in diameter.

*Female organs.* The vagina, a fairly short and narrow canal, runs dorsally to the cirrus pouch. It forms a large (about 0.08 mm.) receptaculum seminis, which lies immediately within the dorsal longitudinal musculature extending to, or but little beyond, the middle of the proglottis. The ovary exhibits a peculiar structure,

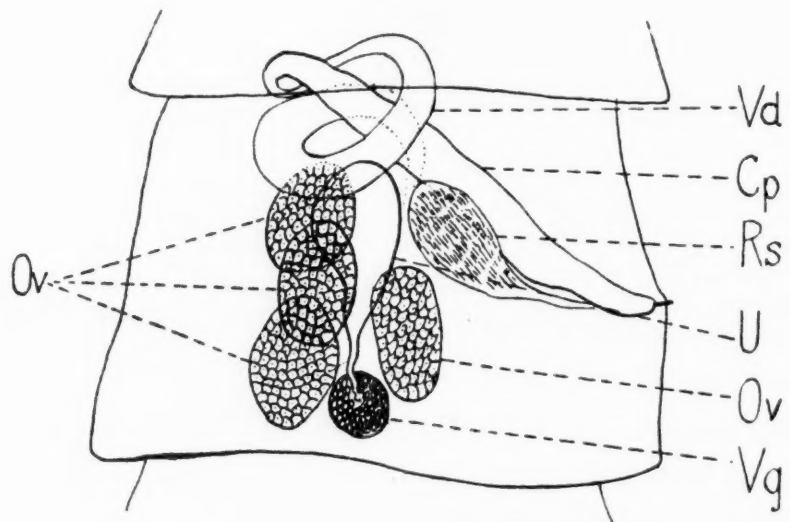


FIG. 4. *D. yorkei*, sp.n. Older segment showing mature female organs. *Cp.*—cirrus pouch; *Ov.*—ovary; *Rs.*—receptaculum seminis; *U.*—uterus; *Vg.*—vittelline gland; *Vd.*—vas deferens.  $\times 170$ .

which, though slightly modified, is characteristic of the two other species also. In the present case it consists of four nearly equal bodies, which are rounded or mostly oval in shape, measuring about  $54\mu$  to  $67\mu$  by  $40\mu$ . One of these ovarian sacs is situated in the poral half, while the three others lie antiporal, i.e., two of them ventral and one somewhat dorsal. Each ovarian sac sends out a thin-walled canal; these unite into a larger, very short oviduct. On the main trunk of the oviduct lies a rounded shell-gland.



A globular vitelline gland of  $29\mu$  diameter is seen in the mid-line towards the posterior margin of the segments.

The young uterus is a thin-walled sac, which lies ventrally in the anterior half of the segments. Growing to a more considerable size, its walls become more distinct; in this stage the female glands disappear suddenly, the whole medulla being occupied by the uterus. In the two or three last segments, however, the wall of the uterus atrophies, the ripe ova filling up the whole space of the proglottides. The rounded ova measure  $54\mu$  in diameter.

I have named this species in honour of Prof. Warrington Yorke, of the University of Liverpool.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

*DILEPIS LEPTOPHALLUS*, sp. n.

Host: *Megapodius brunniventris*, Mey.

Locality: Friedrich-Wilhelmshafen.

The longest worms, when fully developed, measure 80 mm. in length. The scolex is rather similar to that of the former species, its diameter being 0.68 mm. The rostellum is, in its main features, like that of *D. yorkei*, measuring 0.64 to 0.76 mm. in length. It bears on its anterior, knob-like end fifty-two hooks arranged in a double row, those in the anterior row being slightly smaller in size ( $121\mu$  to  $126\mu$ ) than those in the posterior row ( $135\mu$  to  $143\mu$ ). The four suckers are rounded in shape, measuring 0.3 to 0.34 mm. in diameter.

Segmentation begins just behind the scolex. The proglottides are, as a rule, broader than long. The ratio of the length to the breadth varies according to the different stages of contraction. This ratio is in my specimens mostly as 1 : 4-6, so far as concerns the proglottides of the anterior half of the strobila; backwards (caudad) the length increases slightly, the ratio becoming as 1 : 3. The anterior end of the proglottides is usually much narrower than the posterior, which in most specimens shows a distinct thickening.

There is a very well pronounced overlapping, especially in the posterior half of the strobila. Gravid segments are about 2.5 mm. broad and 1.5 mm. long, and exhibit a considerable thickness.

#### ANATOMY.

*Musculature.* The longitudinal muscles consist of bundles, which are arranged in two layers; the internal layer exhibits twenty-six to thirty, the external sixty-six to seventy, mostly oval bundles. The transversal musculature is very poorly developed; mostly it seems entirely absent. The dorso-ventral muscle-fibres are likewise but faintly distinguishable.

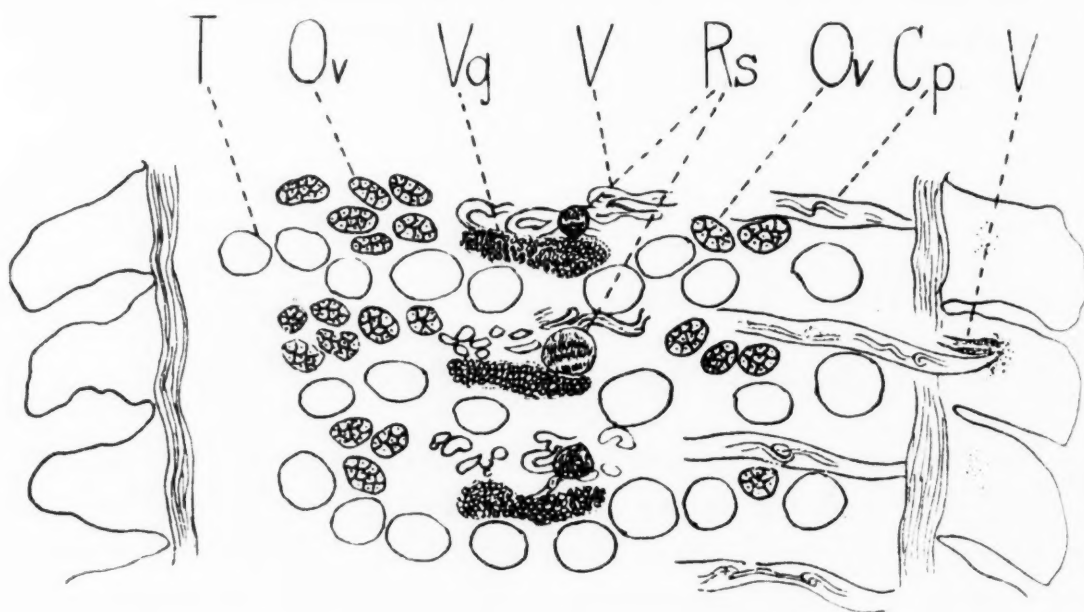


FIG. 5. *D. leptophallus*, sp.n. Longitudinal section of three mature segments. Cp.—cirrus pouch; Ov.—ovary; Rs.—receptaculum seminis; T., testes; V.—vagina; Vg.—vitelline gland.  $\times 100$ .

It is worth noting that in none of the specimens examined could any trace of calcareous bodies be detected.

*Excretory system.* This consists of two pairs of longitudinal vessels, of which only the two wide ventral ones form a transverse canal, this latter being approximately as wide as the main ventral vessels.

*Genital organs.* The openings of the genital ducts are unilateral and lie on the posterior third of the lateral border. The atrium genitale is marked off by a dense network of very small cells of rounded or oval shape.

*Male organs.* The cirrus pouch consists of a very long narrow tube of about 0.68 mm. length and 0.02 mm. breadth. Its position and course depend essentially on the state of contraction of the proglottides, and especially on the progress in development of the genital organs. According to this it runs in normally contracted mature segments from the genital pore to the excretory vessels on the transverse axis, and then turns dorsally, extending a little beyond the middle of the segments. In longer proglottides exhibiting fully developed genital glands, the course of the cirrus pouch is very different from that described above, as (seen in optical longitudinal section) it runs from about the genital pore to the anterior third of the segment parallel to the lateral border, and then turns abruptly to the median part. The cirrus pouch possesses long retractor muscles, which extend beyond the antiporal ventral

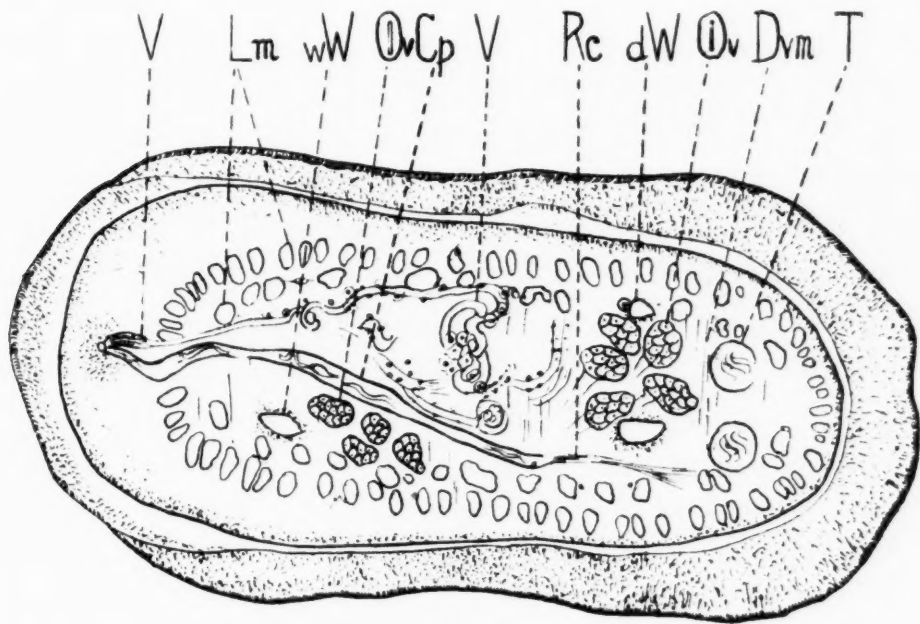


FIG. 6. *D. leptopballus*, sp.n. Transverse section of a mature segment. Cp.—cirrus pouch; Dvm.—dorsoventral muscle fibres; Lm.—longitudinal muscles; Ov.—ovary; Rc.—retractor muscles of the cirrus pouch; T.—testes; dW.—dorsal excretory vessel; wW.—ventral excretory vessel; V.—vagina.  $\times 80$ .

vessel. There is a coiled vas deferens, the coils of which lie in transverse section mainly dorsal, and in a somewhat higher plane than those of the vagina. The cirrus is a very long and slender ( $8\mu$ ) canal, forming usually many coils within the cirrus pouch; in some specimens, in which it was extruded, I could observe that at least on the anterior half it is covered with very minute spines.

*Female organs.* The vagina is a strikingly long duct which runs from the atrium genitale to the mid-part of the medulla, mainly just within the internal layer of the dorsal longitudinal muscles; it forms very large coils, which fill dorso-ventrally the entire central space of the medullary layer. The walls of the vagina are generally thin and covered with rounded cells; immediately before entering the small atrium genitale its walls present a slight sphincter-like thickening. Just in front of the shell-gland there is a rather large rounded receptaculum seminis. The ovary is composed of poral and antiporal lobes, each consisting of distinctly separated groups of acini, the poral lobe having about five groups and the antiporal about ten. The acini are all rather similar in size, measuring about  $43\mu$  across. Each group sends out a thin-walled, narrow canal, all of which, running into a larger one, form a distinct ovarian bridge connecting the two groups of the ovary; from the mid-part of this bridge there arises, at first somewhat dorsally directed, a rather wide oviduct. A similar structure of the ovary exists also in other cestodes, of which the following may here be mentioned:—*Choanotaenia porosa* (Rud.) (see Cohn, 1901), *Ch. gongyla*, Cohn (1901), *Anomotaenia platyrhyncha* (Krabbe), *A. microrhyncha* (Krabbe), and *Ophryocotyle herodiae*, Fuhrm. (1909). A compact, somewhat bean-like vitelline gland lies in the middle of the medulla; it measures 0.14 mm. At the junction of the oviduct and vitelline duct there is a distinct shell-gland of rounded shape.

The uterus appears at first as a rounded, thin-walled sac between both groups of the ovary. It then grows very rapidly, sending out oval diverticula laterally, and usually beyond the excretory vessels as well. All of these sacs then flow together to form a larger one, which at this point has already a more distinct cellular wall. It is an interesting feature that the testes and the receptaculum seminis still persist for a rather long time, the uterus having already occupied transversely almost the whole of the proglottis. In the last few segments the wall of the uterus atrophies, and they are entirely occupied by the ova. The ripe ova measure  $64\mu$  by  $54\mu$  in diameter.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.



*DILEPIS HORVÁTHI*, sp. n.Host: *Megapodius brunneiventris*, Mey.

Locality: Friedrich-Wilhelmshafen.

Among the cestodes collected from this bird I found only a few chains belonging to this new species. With the naked eye it is not easy to distinguish them from *D. leptophallus*. The worms are apparently somewhat shorter than the latter species, the longest specimens measuring 50 mm. The scolex closely resembles in its shape and size that of the former species, being 0.8 mm. in width. The rostellum is still larger, and when retracted it extends with its posterior end to 0.7 mm. behind the posterior border of the suckers. On the anterior knob-like thickening there are fifty-two hooks arranged in a double row; there is but little difference in size between the hooks of the two rows. I found them to be  $99\mu$  in length in the anterior, and  $102\mu$  in the posterior row. The shape, especially that of the anterior hooks, slightly differs from the type shown in the two other species. The suckers are rounded in shape and measure 0.3 mm. in diameter. There is no neck, except in stretched specimens; the segmentation begins a short distance

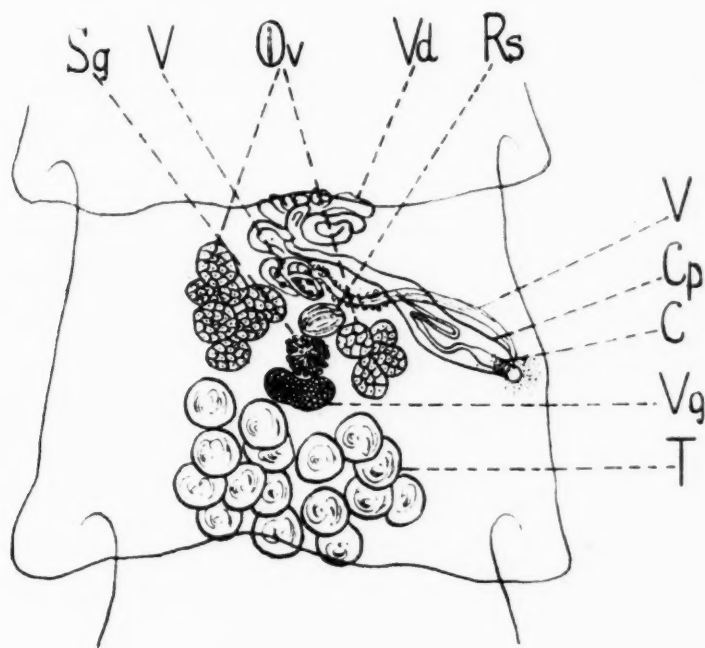


FIG. 7. *D. horváthi*, sp.n. Mature segment. C.—cirrus; Cp.—cirrus pouch; Ov.—ovary; Rs.—receptaculum seminis; Sg.—shell gland; T.—testes; V.—vagina; Vd.—vas deferens; Vg.—vitelline gland.  $\times 80$ .



behind the suckers. The segments in my few chains are usually broader than long, and show otherwise the same features as in *D. leptophallus*. The greatest breadth found in the posterior part of the strobila is 1.5 mm.

Owing to the small number of specimens available belonging to this species, I omitted to sacrifice a chain for the purpose of cutting sections. The following description of the arrangement of the sexual organs is, therefore, based mainly upon worms stained as a whole with boraxcarmin and mounted in balsam.

The genital pores are unilateral, and lie about the middle of the lateral border. It seems that the atrium genitale is of the same extent as in *D. leptophallus*.

The cirrus pouch is a somewhat shorter but wider tube than it is in the former species, measuring about 0.2 mm. in length and 0.04 mm. in breadth; it is usually directed with its long axis obliquely forwards. Within the cirrus pouch is found the rather long cirrus, which at its extremity is distinctly thickened and covered with minute spines. The vas deferens forms many coils, which lie mainly in the middle of the anterior end of the proglottides. The testes lie behind the female organs; it seems that they are less numerous (about fifteen to seventeen) than in the former worm; they measure  $54\mu$  to  $64\mu$ .

The vagina rises anterior to the cirrus pouch; it crosses the posterior end of this organ and then forms apparently as many coils as that of *D. leptophallus*. A rounded receptaculum seminis is similarly present.

The ovary exhibits the same peculiarities as in *D. leptophallus*. If any difference exists in the structure of this organ in both forms, it might perhaps lie in the somewhat fewer number of the ovarian lobes on both the poral and antiporal side.

The vitelline-gland is similar in shape and size to that of the former species.

As gravid segments were not at hand, I am unable to give a suitable description of the uterus and the ripe ova.

The main features which distinguish this species from *D. leptophallus* are:—

1. The shape and size of the rostellar-hooks.
2. The shape and size of the cirrus pouch.

I have named this species in honour of Dr. G. Horváth, Director of the Zoological Department of the Hungarian National Museum in Budapest.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

Among the known representatives of the genus *Dilepis*, there is, I believe, none which exhibits a closer resemblance, so far as the above-mentioned peculiarities are concerned. The type of the hooks might in some respects be likened to those of *D. macrocephala*, Fuhrm. (1908), the scolex and rostellum of which are likewise strong.

All the three species described above are closely related to each other. This is proved by the following features:—

1. The structure of the scolex and its integrate parts (especially the rostellum and hooks).
2. The structure of the male organs, viz., the large cirrus pouch, the reduced number of testes.
3. The structure of the female organs in general and mainly of the ovary.

In philogenetic respects it seems doubtless that the three forms, but particularly *D. yorkei*, are very old representatives of the genus *Dilepis*, and might be perhaps interpreted as a distinct group within this genus.

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# AN ANOPHELES OF THE *MYZORHYNCHUS* GROUP (*Anopheles* *amazonicus* SP.N.) FROM SOUTH AMERICA

BY

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## PLATE IV

Among some material at the Liverpool School of Tropical Medicine, which owing to the kindness of Professor R. Newstead, F.R.S., I was able to examine when home on leave, I was fortunate enough to find a specimen of an anopheline brought by Dr. A. A. Clark from the River Amazon, which not only seemed to be new, but which appeared to be the first instance of an undoubted *Myzorhynchus*, using this term in its restricted sense, recorded from South America, or, indeed, from the New World. On looking up material previously brought by Dr. Clark from this region, Professor Newstead was able to find two other specimens of the same species not quite in such good preservation. With Professor Newstead's kind permission, I give below a description of this species under the name *A. amazonicus*. All three specimens were females, the first mentioned being selected as the type and so labelled in the Liverpool School collection.

*A. amazonicus* closely resembles the Old World species of the group *Myzorhynchus*, and it possesses the ventral abdominal scale tuft on the penultimate abdominal segment which Theobald used to define the genus *Myzorhynchus* when he created it, though it is now known that this character is not present in all the species of the group. In one character, however, *A. amazonicus* approaches the *Arribalzagia* group, which normally, so to speak, represents *Myzorhynchus* in South and Central America. The character referred to is a kink or bend in the costa at the subcostal junction associated with one or more small accessory dark spots in this

position. From the description it will be seen that *A. amazonicus* possesses this feature, though it shows nothing of the other more salient *Arribalzagia* characters such as inflated wing scales, eye-spots on the thorax, abdominal scaling, etc.

A very marked specific feature of *A. amazonicus* is the great length of the anterior forked cell, which measures one-third of the wing length and extends so far inwards that the bifurcation is at the level of the junction of the subcosta with the costa.

*Anopheles (Anopheles) amazonicus*, sp. n.

DIAGNOSTIC POINTS.

An easily identified species characterised by:—

- (1) The wings with pale interruptions on the costa.
- (2) The palps shaggy and without definite bands.
- (3) The hind tarsi dark.
- (4) The femora and tibiae unicolorous.
- (5) The bifurcation of the second longitudinal vein at the same level as the junction of the subcosta with the costa.
- (6) A ventral scale tuft on the penultimate abdominal segment.

DETAILED DESCRIPTION.

♀. A largish dark anopheline of *Myzorkhynchus* appearance; general coloration rather rusty black. Length of wing, 4·4 mm.

*Antennae* with the basal segment dark, free from scales; the second segment with a small tuft of pale and darkish scales on inner aspect; remaining segments free from scales. *Palpi* with the segments, commencing with the rudimentary basal one, measuring respectively 6, 21, 35·5, 21·5 and 16 per cent. of the whole organ. Palpal index (measured from unmounted specimen) 0·7. General appearance of palpi as in *A. umbrosus*, Theo.; densely covered with black erect or semi-erect scales almost to the apex, but with these longer on the basal rudimentary and succeeding segment; apex dark and organ without obvious pale bands, though one or two light scales are present at the apices of segments three and four difficult to see except in certain lights. *Labium* black scaled, the



scales somewhat erect over basal half; labellae darkish. *Clypeus* dark, bare. *Head* with the frons and vertex with small very narrow white scales, less prolonged than usual. The pale area smaller in extent than usual; including the area of narrow scales in front and about the same extent of the ordinary upright scales behind this. Occiput with dark erect truncate scales of ordinary anopheline type, extending below level of neck. Some broad white scales beneath, between the eyes, gular chaetae black.

*Prothoracic lobes* with dense tufts of black erect scales and numerous chaetae. *Prosternal hairs* about four. *Mesonotum* of uniform coloration, dull brown, the bare spaces, etc., not conspicuous; chaetae inconspicuous and presence of median series doubtful. The surface clothed with light coloured hairs, scantily but fairly uniformly distributed over the dorsum, including the fossae. Anterior promontory with a smallish area medially of long curved, pale scales, not forming a conspicuous feature; laterally, and extending about half way to the lateral angular process of the mesonotum, are rather conspicuous erect pale spatulate scales. *Scutellum* with about twenty-four large hairs and a second line of two additional hairs on each lateral lobe; scattered smaller impressions (scales or hairs) medially. *Spiracular hairs* about two. *Pre-alar hairs* about eight.

*Wings* with the length 4.44 mm. and the greatest breadth 1.05 mm. Base to subcostal junction 0.67, anterior forked cell 0.33, posterior forked cell 0.19 of the length of the whole wing. Forked cell index 1.8. The anterior forked cell unusually long, and the bifurcation of the second longitudinal vein so far towards the base of the wing that it is on a level with the junction of the subcosta with the costa.

The wing markings, as a whole, are rather diffuse, the pale areas not being very distinct, whilst there is an admixture in places of pale and dark scales. Costa mainly dark, but with the following pale areas: a minute one near base, a well marked one at about the junction of the inner with the middle third of the wing length; a comparatively large one just internal to the subcostal junction, a somewhat smaller one actually at the subcostal junction and one at the apex of the wing not quite reaching to the point of junction of the first longitudinal with the wing margin. The most

characteristic feature of the costal markings is the presence of the small accessory dark spot, involving the costa only, which lies between the two pale areas in the region of the subcostal junction. In this position there is also seen a slight but distinct bend or kink in the costa, as in species of *Arribalzagia*. The *first vein* is marked as the costa, but with additional pale areas at the base and at the accessory sector. The *second vein* has the stem mainly pale scaled, with dark scales at the cross-vein and just distal to its origin; the upper branch has a small pale spot just external to the middle, and the lower branch an indistinct one somewhat internal to this; the branches are also pale where they join the wing margin, though the fringe itself here is dark. The *third vein* is mainly dark, but has light scaled areas, separated by a conspicuous small dark spot, on its basal portion. The *fourth vein* stem is dark, with white scaled areas near the base and proximal to the cross-vein. The branches are mainly dark with pale scales forming one (or two) small spots on the upper branch. The *fifth vein* has the stem with mixed dark and pale scales, the anterior branch with pale patches near the base and beyond the cross-vein, the posterior branch with pale (mixed with dark) scales on its proximal and dark scales on its distal half. The *sixth vein* has alternate dark and light portions (four pale and four dark areas). The *fringe* is dark from the apical costal spot to the space between the veins 3 and 4'1 where there is a light spot. There is another somewhat indefinite pale spot between 4'2 and 5'1. The remainder of the wing fringe is too rubbed in all the specimens for description.

Except for the spots on the costa and on vein 2'1, the scales of the under surface are all dark. The wing membrane is stained, but is lighter at some of the pale scaled areas.

The scaling of the wing shows the normal arrangement, but the truncated squames of the median series are very inconspicuous owing to the development of the laterals, whilst these latter and the plume scales of the reverse side of the veins approach each other in character so closely that they are scarcely to be distinguished. The general effect is a heavy scaling with rather uniform large obovate scales. The squames show from seven to nine striations, the laterals ten to eleven, and the plumes usually nine striations, but some slightly broader plumes are present on the fourth vein (upper

surface), where they may show as many as twelve striations. This is the position where the large inflated scales of *Arribatzagia* occur.

The *coxae*, in the case of the anterior pair, have black scales basally and anteriorly, also posteriorly and apically. The middle and posterior pair appear devoid of scales. The anterior *trochanters* with black and white scales, the middle and posterior apparently devoid of scales. *Femora* of the anterior pair moderately dilated in inner half. The femora and tibiae of all the legs without definite markings, except that there is a lightish triangular spot on the mid-tibia apically. Tarsal segments of all the legs dark, unicolorous, but with the apices of segments one, two and three narrowly pale, four and five being dark.

The abdomen with hairs only, except ventrally. Cerci with hairs only. Ventral surface with hairs, except medially, where on segments four to seven, somewhat nearer the posterior than the anterior border of the segment, are small patches of white scales, the number of scales increasing up to the patch on the seventh segment. On the seventh segment, posterior to the white scales, is a prominent projecting tuft of black scales.

HABITAT, etc. The specimens were collected by Dr. A. A. Clark on his journeys up and down the Amazon. The type was labelled 'A. A. Clark, River Amazon, June, 1915.'

*A. amazonicus* is distinguished from *A. vestitipennis*, Dyar and Knab, to which it has some resemblance, by the absence of speckling of the femora and tibiae, by the tarsal markings and by differences in the wing markings. It is distinguished from *A. crucians*, Wied., by the costal and palpal markings, from the *Myzorhynchellas* by the uniform colour of the hind tarsi, and from *A. peryassui*, Dyar and Knab, by the absence of eye-spots on the thorax, etc.

The only species about which some doubt must remain is *A. mattogrossensis*, Lutz and Neiva. The description of *A. mattogrossensis* given by Lutz and Neiva (1911) corresponds in a number of respects with the species now described. But the wings are described as 'rather dark, especially on the costa, where there are two spots lighter in colour, greyish yellow; there is a band, whitish-yellow, transversal and sub-apical, formed by a group of cream-coloured scales; there are others distributed in a somewhat irregular manner upon the longitudinal veins, scarcely distinguishable by the

naked eye.\* As there are three quite distinct spots on the costa of *A. amazonicus*, the correspondence here would not seem to hold good. The description also says nothing about a ventral tuft on the penultimate segment, though the ventral surface of the abdomen is described as 'having traces of elongate scales, narrow and rather long.† In *A. amazonicus* the scales, other than those forming the tuft, are few in number, and would scarcely be described in the words used in the description of *A. mattogrossensis*. Unfortunately the description given of *A. mattogrossensis* is rather meagre, and I am unaware of any other reference to this species giving any further particulars, whilst the type is presumably in South America.

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\* The original passage reads, 'Azas bastante escuras, principalmente na costa, onde ha duas manchas de côr mais clara, amarelo-pardacenta; ha um risco branco-amarelado transversal e subapical, formado por um agrupamento de escamas de côr *creme*; ha outras, distribuidas de modo um tanto irregular, sobre as nervuras longitudinais, que apenas se distinguem a olho nú.'

† 'na ventral ha vestijios de escamas alongadas, estreitas e pouco compridas.'





## EXPLANATION OF PLATE IV

- Fig. 1. Camera-lucida drawing of wing of *A. amazonicus*. The scales are not shown, but the positions of the dark and pale scaled areas on the veins are indicated by shading.
- Fig. 2. Truncated squames of the median series (obverse scaling).  
*a.* Costa internal to subcostal junction.  
*b.* First longitudinal, basal portion.  
*c.* do. at level of subcostal junction.  
*d.* Stem of vein 5.
- Fig. 3. Lateral squames (obverse scaling).  
*a, b, c.* As in fig. 2.
- Fig. 4. Scales *in situ* on anterior branch of vein 5.  
*m.* Median.  
*l.* Lateral.  
*pl.* Plume scales of reverse side of vein seen through the wing membrane. Vein 5 is a normal vein, *i.e.*, the squame scales are uppermost.
- Fig. 5. Plume scales of reverse aspect of veins.  
*a.* Costa external to subcostal junction.  
*d.* Stem of vein 5.  
*e.* Stem of vein 4, upper surface. Vein 4 is a reverse vein, *i.e.*, the squames are beneath and the plume scales uppermost.
- Fig. 6. Ventral view of terminal portion of abdomen, showing scale tuft.

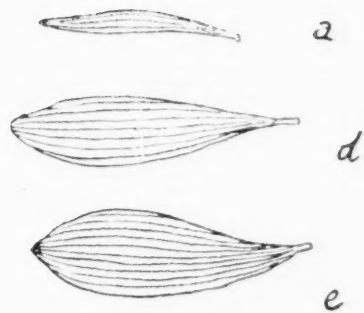
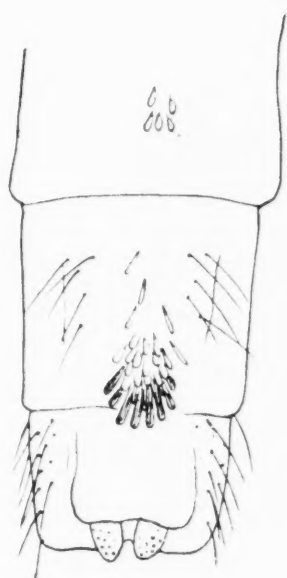
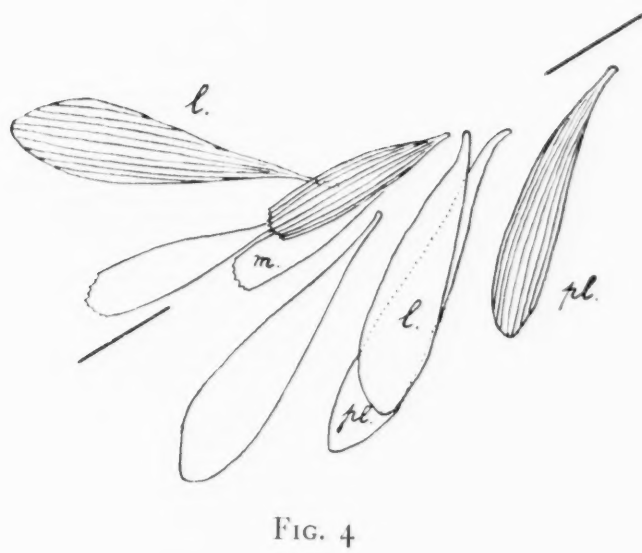
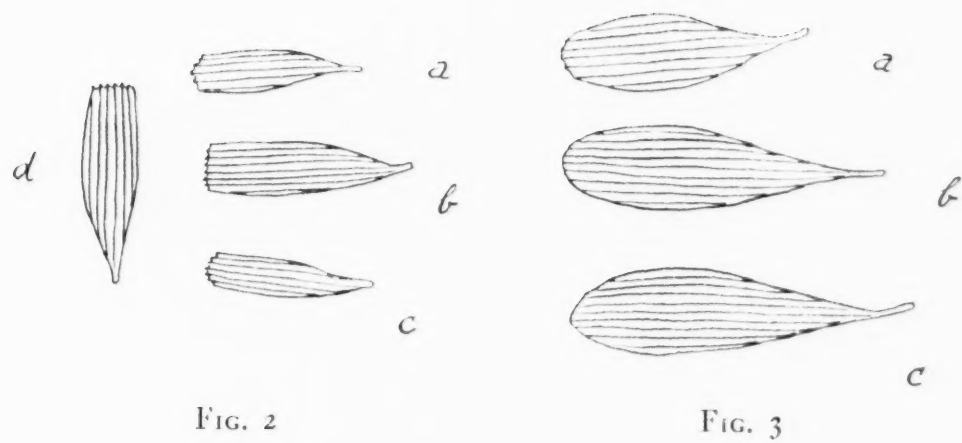


FIG. 1



# THE ETIOLOGY OF BLACKWATER FEVER

BY

B. BLACKLOCK

*(From the Sir Alfred Lewis Jones Research Laboratory, Freetown)*

*(Received for publication 5 February, 1923)*

Marchiafava and Bignami (1894), in referring to malarial poisoning, say: 'We may mention those morbid states which are developed after the malarial (parasitic) infection has passed away; for instance, the post-malarial fevers, the delirium, the post-malarial haemoglobinuria.' Mannaberg (1894) emphasises the fact that Kelsch and Kiener proved that in every severe case of malaria, even in every malarial cachexia, haemoglobinuria may be observed, and calls attention to the effects of lesions of the renal epithelium as pointed out by Bignami. These observers recognised haemoglobinuria as a relatively common complication or sequela of malaria, and it is of interest that they recognised it as a fact that, when haemoglobinuria develops after malaria, the parasites have disappeared from the peripheral blood.

Later quinine was added as a supplementary cause of the attacks of haemoglobinuria. Of the numerous more recent theories of the cause of blackwater fever with which we are chiefly concerned here, the first is Manson's, who stated that blackwater fever is a disease by itself, separate from and not dependent upon malaria; the second, which is an advance on this, claims for blackwater fever that it is produced by a living organism. Further suggestions, still largely in the realm of speculation, have been made by which this or that form of parasite is stated to be the cause of blackwater fever. Some of these parasitic theories we shall have the opportunity of mentioning later.

Although it is thirty years ago since Manson promulgated the theory that blackwater fever is a disease entity, and although numerous observers since have attributed to different organisms the

credit of being the cause of it, the specific parasite which gives rise to blackwater fever is still undiscovered. The older malaria, malaria-quinine and similar theories have suffered much at the hands of critics, but to fill their place little of definite value has been produced. The tendency has been to admit that while haemoglobinuria occurs as the result of malaria, and quinine and other drugs, yet apart from these haemoglobinurias, and separable from them even when, in the tropics, they occur in chronic malaria cases who have been taking quinine, there is a definite recognisable condition of haemoglobinuria which constitutes the main sign of blackwater fever.

Castellani and Chalmers (1919) differentiate the haemoglobinurias which may occur in the tropics into three groups: Symptomatic, Toxic and Specific. Under symptomatic they put haemoglobinuria occurring in the course of malaria, Raynaud's disease, acute specific fevers, and after severe burns. The toxic group includes haemoglobinuria resulting from the administration of quinine and its salts, chlorate of potash, antipyrin, carbolic acid, and naphthol, or from vegetable substances such as *Vicia faba*. The specific group comprises blackwater fever and paroxysmal haemoglobinuria. At first glance such a classification appears of assistance to those who are likely to come in contact clinically with blackwater fever cases, eliminating from the sphere of blackwater fever those confusing elements which are introduced if what is in reality a symptomatic or toxic haemoglobinuria is erroneously attributed to a specific disease.

Before accepting this classification, however, it may be well to consider in some detail the signs and symptoms by which these varieties of haemoglobinuria are said to be distinguished from one another. In Table I, compiled from these authors, are given in comparative columns the signs and symptoms under each form of haemoglobinuria, the symptomatic group being represented by malaria, the toxic by quinine, and the specific by blackwater fever.

Reviewing the table, it is worthy of note that the signs and symptoms of quinine haemoglobinuria resemble those of an attack of blackwater fever but are not so severe, and that jaundice is specially mentioned as being slight or absent in the former condition. The malaria group is allotted six positive signs and symptoms, which



TABLE I

Comparison of signs and symptoms of Tropical Haemoglobinurias.

Symptomatic	Toxic	Specific
Haemoglobinuria in malaria, Raynaud's Disease, Acute Specific Fevers and after severe Burns	Haemoglobinuria caused by Quinine, Chlorate of Potash, Antipyrin, Carbolic acid, Naphthol and <i>Vicia faba</i>	Haemoglobinuria in Blackwater Fever and Paroxysmal Haemoglobinuria
Malaria	Quinine	Blackwater Fever.
1. Haemoglobinuria 2. Fever 3. Rigor 4. Vomiting 5. Prostration 6. Anaemia  Negative.—Rarity of severe Jaundice	Resemble those of an attack of Blackwater Fever, but are not so acute. Jaundice slight or absent.	1. Haemoglobinuria 2. Fever 3. Rigor 4. Vomiting 5. Intense weakness 6. Anaemia <i>Additional.</i> (a) Anorexia (b) Headache (c) Pains Back and Legs (d) Nausea (e) Diarrhoea (f) Thirst (g) Constipation (h) Jaundice (i) Hyperpyrexia (j) Coma

also occur in the quinine and blackwater fever columns. Additional signs and symptoms are enumerated under blackwater fever, and these evidently apply also to the quinine group, since the signs and symptoms of the latter are said to resemble those of an attack of blackwater fever but are not so severe. The value of these additional signs and symptoms as a means of distinguishing the blackwater and quinine groups on the one hand from the malaria group on the other, appears to be entirely discounted by the fact that these signs and symptoms are all, without exception, adduced by the authors in their foregoing description of one or other form of the malaria infections.

In the analysis of the differential diagnosis we find ourselves reduced to the following:—

- (1) In the malaria group, the rarity of severe jaundice.
- (2) In the quinine group, the relative lack of severity of the symptoms and the fact that jaundice is absent or slight.

*Jaundice.*

The patient whose chart is given below and who died of blackwater fever presented, some weeks before the date of the commencement of the chart, slight jaundice, which passed off in a day; he had a similar slight transient jaundice a week before his fatal attack of blackwater fever. At the time of the second attack of mild jaundice he had subtertian parasites in his blood in small numbers, and he had also taken quinine irregularly. To what, then, are we to attribute these mild attacks of jaundice? To haemolysis from malaria, or quinine or blackwater fever? It appears legitimate to assume that they were a manifestation of the same causes of haemolysis as produced the marked attack of blackwater fever. It is of importance to note that in this case during the fatal attack the jaundice was not of an intense kind. Deep jaundice, again, is known to occur in malaria; in fact, many authors include the 'yellow fever-like type' of malaria in their description. One of the cardinal signs of this type is deep jaundice.

From a study of this table of differential aids, one must conclude that although it may in the future be possible to distinguish accurately between a malaria, a quinine and a blackwater haemoglobinuria, this cannot by such aids be done to-day; the attempt at differential diagnoses on such slender evidence as the degree of jaundice and the severity of the symptoms is unscientific. It is commonly stated that blackwater fever is, owing to its severity, a condition which leaves no doubt in the mind as to the diagnosis. But if blackwater fever is a disease which presents itself in an acute form, and in an acute form only, then it is, indeed, a disease *sui generis* and incomparable with any other known disease.

Stephens' views on blackwater fever are quoted by the authors: 'Blackwater is not a disease *per se*, but rather a condition of blood in which quinine, other drugs, cold or even exertion, may produce a sudden destruction of red cells. The condition is produced only by malaria, and generally by repeated slight attacks, insufficiently combated by quinine. In such cases of chronic malaria, *i.e.*, in those suffering from anaemia, with repeated attacks of fever and repeated doses of quinine, blackwater fever sooner or later almost certainly supervenes, at least in tropical climates.' The authors' comment upon Stephens' account is as follows:—'These statements are too

sweeping if genuine blackwater is meant, otherwise the home of the disease would be Ceylon, whereas it is so rare that we have never heard of a genuine non-imported case; for in this island there are Europeans and natives with just the conditions required by Stephens, and yet they do not develop blackwater fever, because the only two cases which we have met with or heard of in Ceylon in twelve years were most probably cases of quinine haemoglobinuria. On the other hand, Stephens' remarks are correct if applied to quinine haemoglobinuria.' The last sentence of this criticism is important. If it be a fact that in Ceylon there are Europeans and natives with just the conditions required by Stephens, and if it be a fact that Stephens' remarks are correct if applied to quinine haemoglobinuria, how are we to explain the low prevalence of quinine haemoglobinuria in Ceylon, *i.e.*, two cases in twelve years?

The observation was made in this case of the occurrence of transient jaundice on two occasions before the severe attack of blackwater fever, malaria parasites being present on the second occasion; these preliminary attacks of jaundice may have represented the occurrence in the blood of—in a less degree—the same changes produced by the same cause as was active during the attack. The probability of such mild haemolytic attacks is great and they are easily overlooked by the patient, as they were in this case. It is also unlikely that anything short of a severe attack will attract the attention of the patient to his urine, and if the attention is not drawn to the urine, it is certain that under the conditions of life in such places as Africa a person will frequently fail to notice that his urine is abnormal. In order to observe even considerable degrees of haemoglobinuria, it is necessary to examine the urine in a suitable vessel in a good light, precautions not usually possible for patients living under the conditions which prevail in places where blackwater fever occurs. I would suggest, then, that closer investigation will reveal the fact that haemoglobinuria occurs frequently in the tropics without being observed, and that still more frequently haemolysis with slight jaundice occur without noticeable haemoglobinuria, and that these conditions are in fact frequently due to the same causes as blackwater fever and are mild forms of the same condition. Even in England, one has seen a case who was walking about and was unaware of the fact that he was passing haemoglobin in the urine in quite noticeable quantity.

Short of 'blackwater' fever, which represents a gross haemoglobinuria, there must be many degrees of haemolysis, haemoglobinaemia and slight haemoglobinuria produced by exactly the same agencies as produce 'blackwater.' For such cases I would suggest that the term 'blackwater' fever is not sufficiently comprehensive. We require a term for such conditions to indicate that the process of haemolysis has not produced such a degree of haemoglobinaemia as to result in the passage of haemoglobin in the urine.

Numerous suggestions as to the nature of the causal parasite of blackwater fever have been made during the last thirty years. Protozoa, bacteria, spirochaetes and chlamydozoa are represented among the suggested parasites. The suggestion of Sambon that blackwater fever might be due to a piroplasma-like parasite has been accepted by some, and there are many points of resemblance between this condition in man and piroplasmosis in animals. Dudgeon (1920) injected sterilized urine from cases of blackwater fever—obtained during the period of haemoglobinuria—into animals, without producing any ill-effects. This observer mentions as a possibility that the disease may be caused by a filter passer.

#### *Experimental Inoculation of Blackwater Fever Blood*

In order to throw some light upon this important question of whether or not there is a specific parasite or enzyme which causes blackwater fever, an experimental inoculation was performed. Blood was taken from the patient in the middle of what proved to be a fatal attack of blackwater fever, and was injected into a healthy European. The blood was withdrawn from a vein in the arm into a syringe containing citrated saline solution (2 per cent. Sod. cit. and 0.85 per cent. Sod. chloride, equal parts) and was injected in two portions into the recipient. The proportion of blood to citrate saline solution was three to one, and of this about 10 minims was injected deep into the region over the deltoid muscle at 3.45 p.m. and 2 c.c. into the same region at 4 p.m. The recipient had previously had malaria, the last infection being of the subtertian variety, but he had been free from relapse for over eighteen months and had taken no quinine for over eight months.

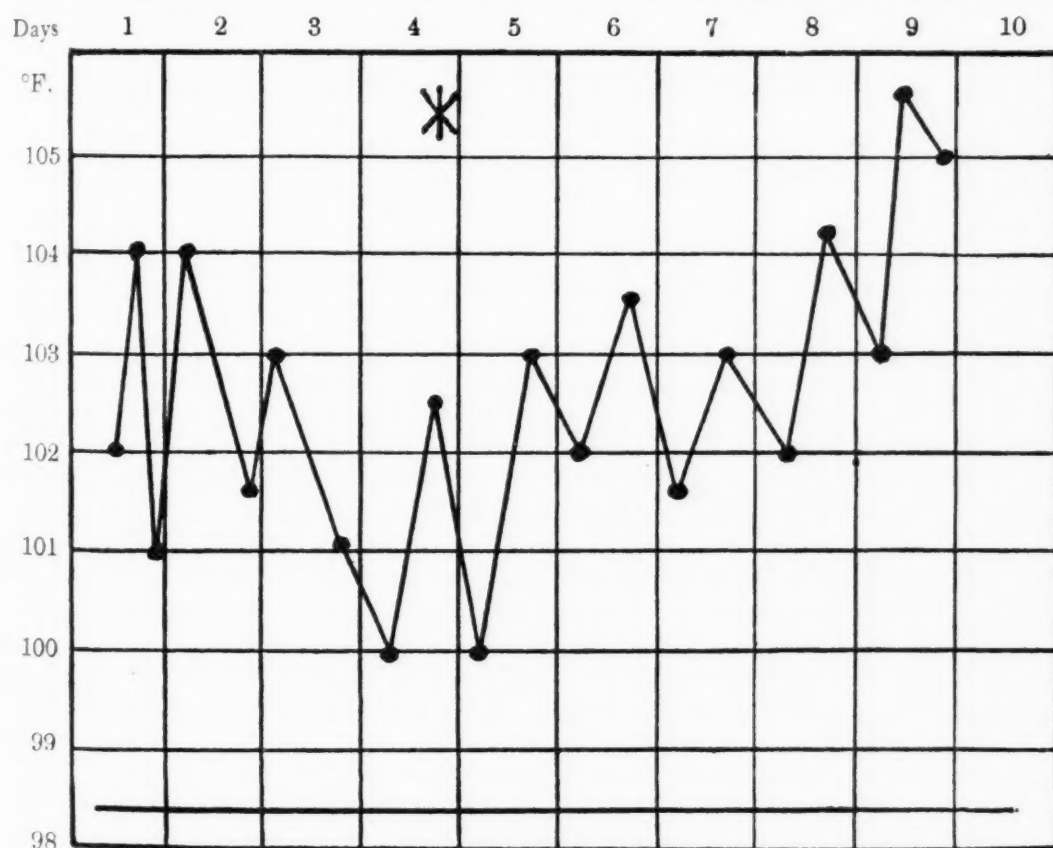


There was no local or general reaction immediately following the injections. Quinine bihydrochloride was administered orally in order to obviate infection with malaria. The dates, times and doses were as follows :—

December 4	...	10 grs.	...	4 p.m.
	...	5 grs.	...	8 p.m.
	...	5 grs.	...	10 p.m.
December 5	...	5 grs.	...	9 a.m.
	...	5 grs.	...	1 p.m.
		<u>30 grs.</u>		<u>in 21 hours.</u>

It might be argued that the doses of quinine taken might be capable of killing the parasite causing blackwater fever. Against this we have the record of numerous cases of blackwater fever in which even large doses of quinine failed to abate or ameliorate the condition. Also in the fatal case in question, quinine was administered by intramuscular injection on the sixth and seventh days of the disease, 21 grains in all, without influencing the temperature or improving the general condition.

Appended is the chart of the case giving the highest and lowest temperatures recorded each day. It will be observed that at the



Temperature Chart of fatal case of Blackwater Fever.

\* Time at which injection was made.



time of injection the temperature of this patient was over  $102^{\circ}\text{F.}$ , and that it remained high till his death five days later. It seems likely that if there was an infective agent it should have been present in the blood on the day of inoculation. Sources of fallacy include the possibility that the parasite of blackwater fever is never present in the blood at all; that it is present in such small numbers that the amount injected did not include the organism; that the parasite is present only for one or two days at the commencement of the disease; that it has an unusually long incubation period; or that the subject of inoculation was immune.

#### *Result of inoculation*

No immediate nor late effects were noted as the result of the inoculation. Parasites were not found in the blood, nor was there any rise of temperature nor haemoglobinuria observable during a period of two months.

These facts appear to me to militate against the specific parasitic theory of the etiology of blackwater fever.

### **SUMMARY AND CONCLUSIONS**

1. The term 'Blackwater' Fever, being applicable only to conditions in which haemoglobin is present in visible quantity in the urine, is too restricted.
2. The importance of pre- and post-haemoglobinuria states which are inherent parts of the disease, is apt to be lost sight of owing to the exclusive use of the term 'Blackwater' Fever. Some such term as 'Occult' or 'Subliminal' Blackwater Fever might be used to express these conditions.
3. A differentiation of Tropical Haemoglobinurias into Malaria, Quinine and specific Blackwater types is not possible merely on the basis of the presence and degree of jaundice, or on the relative severity of the signs or symptoms.
4. The existence of a parasitic cause of Blackwater Fever has been frequently suggested; an experimental human inoculation, with

blood from a severe case of Blackwater Fever which ended fatally, elicited no evidence in favour of the existence of such a parasite after an observation period of two months.

#### ACKNOWLEDGMENT

I have to thank Dr. J. Y. Wood, W.A.M.S., for performing the inoculations.

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# A NEW SPECIES AND A NEW VARIETY OF *CULEX* FROM THE BELGIAN CONGO

BY

A. M. EVANS

*(Received for publication 9 February, 1923)*

The new species and variety of *Culex* described in the present paper occurred among two small collections of mosquitoes from the Belgian Congo, which were sent by Dr. Severin to this School for determination.

## *Culex moucheti*, n. sp. (fig. 1).

This species is named in honour of its discoverer, Dr. Mouchet.

*Head.* Scales bordering the eyes white, flat below, and becoming gradually narrower as they approach the vertex. Upright forked scales golden yellow in front, very dark brown behind, intermediate ones golden with very narrow black tips. Narrow curved scales silvery white. Bristles projecting over vertex golden yellow. Scales of proboscis, female palpi, and scales and hairs of male palpi dark sepia.

*Thorax.* Prothoracic lobes with whitish scales. Mesonotum dark brown with bronzy scales, apparently discoloured by dampness, and blackish bristles. Pale whitish scales on ante-scutellar space, scutellum, and medially just behind the head. Pleurae with green integument. Lower mesepimeron of type with one bristle socket on left side.

*Abdomen.* Tergites II to VII entirely covered with dark sepia scales, except at side of tergites IV to VII, where basal triangular patches of whitish scales may occur; these very small, except on segment VII. Tergite VIII with large irregular patch of whitish scales above. Sternites entirely whitish scaled.

*Legs.* Vestiture chiefly blackish-brown. Femora pale beneath; middle tibiae and tarsi in some specimens with whitish scales beneath, extending for the whole part of the length of the segments.

*Wings.* Plume scales on third vein ligulate, squames on costa and first vein with six to eight striae. First fork cell slightly longer than its petiole, its base distal to that of second fork cell.

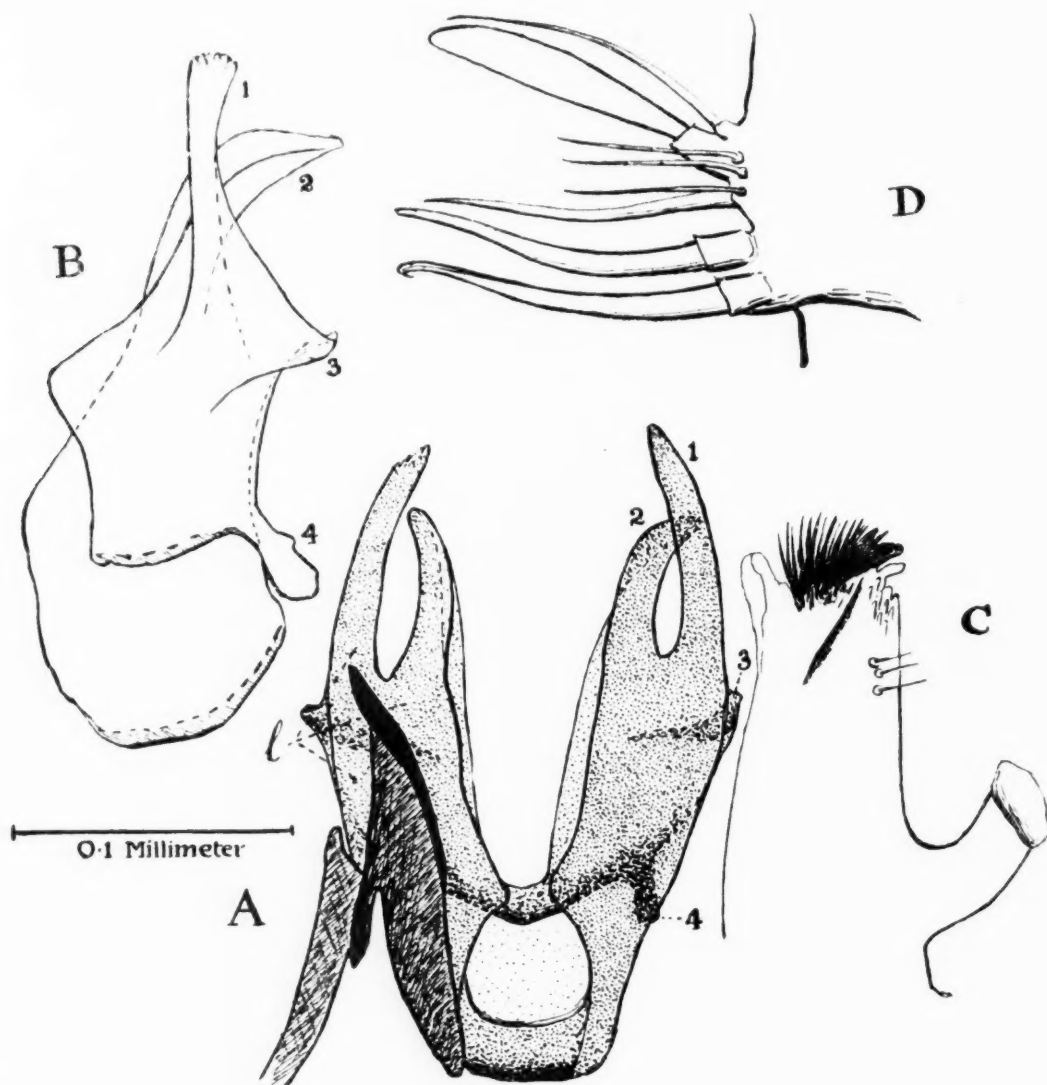


FIG. 1. *Culex moucheti*, n.sp. Male hypopygium. A—phallosome, ventral aspect with left parameral plate and part of basal plate; 1, 2, 3, and 4—processes of phallosome, *l*—left side. B—left half of phallosome, postero-lateral aspect somewhat distorted, 1, 2, 3, and 4, as in A. C—tenth sternite, ventral aspect. D—lobe of side-piece.

*Male hypopygium* (fig. 1). Lobe of side-piece as in fig. 1, *d*. The occurrence of only one moderately stout rod between the leaf and the pair of very stout rods a constant feature in the five specimens examined. *Phallosome* (Christophers, 1922) or mesosome



(Edwards, 1920) (fig. 1, *a* and *b*) of simple structure, chitin of walls thin and uniform except in region of basal tooth (4). Each half of phallosome with two long terminal processes (1 and 2), the longer weakly serrated distally, a short pointed process (3) projecting dorso-laterally, and a chitinised tooth (4) at basal angle of dorsal wall. Tenth sternites as in fig. 1, *c*.

Type ♂, six co-type ♂♂, and three co-type ♀♀ from Stanleyville, Belgian Congo, 1922, Dr. Mouchet.\*

This species is one of the *pipiens* group of *Culex*, as defined by Edwards (1922). The hypopygial characters point to a relationship with *Culex pipiens*, L., and *C. triflatus*, Edwards, but the absence of abdominal bands and of lines of white scales beneath the last two male palpal segments would seem to indicate affinities with the *deccens* series of the group.

*Culex annulioris* var. *congolensis*, n. var.

Male resembling typical *C. annulioris*, Theo., in hypopygial characters, banding of palpi, proboscis and tarsi, and scaling of thorax, but with *abdomen entirely dark scaled above*, the median basal and lateral apical white markings characteristic of *annulioris* being entirely absent.

Type and co-type ♂♂ from Leopoldville, 1922, Dr. Duren.

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\* Since going to press two further ♂♂ of *Culex moucheti* have been received from Buta, Belgian Congo, November, 1922, Dr. Mouchet.



## REPORT ON SLEEPING SICKNESS IN EKET DISTRICT, SOUTHERN NIGERIA

BY

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*(Received for publication September, 1922)*

With the object of ascertaining the present position of sleeping sickness in the Eket District, Southern Nigeria, a tour of the whole district was made during April and May, 1922.

The route followed was from Oron to Awa, thus passing through the centre of the Eket District. The main towns at which I stopped and made enquiries were Oyubia, Ikorubo (site of old sleeping sickness camp), Eket and Awa, whilst numerous small, so-called villages were also inspected.

For the sake of clearness, and to avoid confusion, this report is divided into three parts:—

- (1) Result of cases recorded in the year 1912.
- (2) Result of cases not recorded in 1912, but who gave definite information that they had been inmates of the camp.
- (3) General enquiry into trypanosomiasis in the Eket District during the months of April and May, 1922.

The whole investigation has been most difficult, the people showing the greatest reluctance to impart any information on the subject, and it was only after prolonged interviews, which were most wearying, that eventually the information detailed below was obtained. Very great assistance was given by the District Officer, as well as by members of the Qua-Ibo Mission.

## PART I

In Macfie's Report (1915) on sleeping sickness in the Eket District in 1913, the following passage occurs:—

‘During the sixteen months in which sleeping sickness has been under investigation, two hundred and twenty-two cases have been identified in which the trypanosomes have been demonstrated. In addition one hundred and fourteen cases have been met with presenting some of the clinical features of the disease, but in which parasites have not actually been found. There can be little doubt that the majority of these were cases of trypanosomiasis.’

The nominal roll of cases made by Macfie has unfortunately been lost, but I have assumed that the two hundred and twenty-two cases noted by him include eighty-nine definitely recorded cases, lists of which, either in manuscript or typescript, were compiled in 1912 by Foran and Gray, with a signed statement to the effect that all had been diagnosed microscopically.

Of these eighty-nine cases I was able to trace thirty-five, details of which are noted in Tables I and II. The remaining fifty-four cases have not been traced and nothing is known about them.

TABLE I.

Cases recorded in 1912 by Dr. Foran and Dr. Gray, and which are still alive.

Name	Age	Sex	Residence	When recorded	Remarks
Udo ... ..	9	M	Edem Idem Ekpota	8.8.12	Seen April, 1922. Healthy. No glands, but thickening on sides of neck which shows scars.
Usundurus ...	9	F	Ikorubo	7.8.12	Not seen. Is reported to be alive and well.
Enoesiet ... ..	adult	M	Afaha Eket	8.8.12	Seen 1922. Thickening on neck, with scars; no glands to be felt; strong and healthy.
Obotnt Ekim ...	12	F	Edem Idem Ekpota	8.8.12	Seen 1922. Strong and healthy; no glands to be felt.
Etok Awa ... ..	adult	F	Ikorubo	12.8.12	Seen 1922. Healthy; no glands to be felt. Slight scars on neck.
Adia ... ..	6	F	Idikpa	15.8.12	Seen 1922. Healthy; no glands to be felt. Slight scars on neck.

TABLE I—continued

Name	Age	Sex	Residence	When recorded	Remarks
Ekanem ...	11	M	Ikorubo	16.8.12	Not seen. Left the country apparently well.
Usoanwan ...	adult	F	Ikotesiokong	20.8.12	Seen 1922. Healthy. One small, very hard gland on neck.
Umwa Etok ...	7	F	Ikorubo	21.8.12	Seen 1922. Healthy; no glands or scars.
Amame ...	10	F	Ikotesiokong	24.8.12	Not seen. Alive and well, but refused examination.
Mama ...	17	F	Ikotesiokong	24.8.12	Not seen. Alive and well, but refused examination.
Okposen ...	adult	M	Ikotesiokong	27.8.12	Seen 1922. Healthy; scars on neck; three very small hard glands to be felt.
Adiah Ansudo ...	9	F	Ikotoquot	28.8.12	Not seen. Said to be alive and well.
Akpanitauwen ...	12	M	Ikorubo	28.8.12	Seen 1922. Healthy; one small gland on neck freely moveable.
Owoimaha ...	18	M	Afaha Eket	31.8.12	Seen 1922. Scars and thickening of neck.
Esoena ...	adult	M	Ikotesiokong	2.9.12	Seen 1912. No glands, but considerable thickening both sides of neck.
Peter Nsooyo ...	adult	M	Ekpenobo	11.9.12	Seen 1922. No glands, but scars both sides of neck. Strong and healthy.
Ntanwoo ...	14	F	Efrievo	13.9.12	Seen 1922. Healthy; a few very small hard glands felt. Gland puncture refused.
Samuel Akpanuso	adult	M	Ikorubo	24.9.12	Seen 1922. Healthy; a few small glands to be felt; gland puncture negative.
Eya ...	17	F	Effoe	22.9.12	Seen 1922. Healthy; no glands; some thickening.
Ema ...	9	F	Akai	20.5.12	Seen 1922. Healthy; scars and thickening, but no glands to be felt.
Wilson Akpan ...	9	M	Ikorubo	?	Seen 1922. One small gland felt; gland puncture negative.
Adiaha Esein ...	13	F	Inoiya	28.5.12	Seen 1922. Healthy; some scars and thickening of neck.



TABLE II.

Cases recorded in 1912 by Dr. Foran and Dr. Gray, and which have died.

Name	Age	Sex	Residence	When recorded	Date of Death	Alleged cause of Death
Adiansun...	18	F	Ikorubo	15.8.12	1918	Influenza.
John Opan ...	adult	M	Ikorubo	17.8.12	1921	Small pox.
Adong ...	9	F	Ikotesiokong	17.8.12	1916	Sleeping sickness.
Samso Okpa ...	12	M	Ikotesiokong	20.8.12	1921	Small pox.
Ikotumoanwan ...	9	M	Ikotoquot	22.8.12	1915	Sleeping sickness.
Edikpoi ...	18	M	Ikorubo	27.8.12	1912	Sleeping sickness.
Udouqua...	13	M	Ikotesiokong	27.8.12	1912	Sleeping sickness.
Eno ...	8	F	Okong	27.8.12	1918	Influenza.
Ekpo Awan ...	8	M	Ikotodiong	2.9.12	1918	Influenza.
Esukoku ...	12	M	Ikotesiokong	7.9.12	1915	Sleeping sickness.
Ikpeisak ...	11	F	Ikotesiokong	20.9.12	1915	Sleeping sickness.
Idimedoho ...	15	M	Ikotekong	23.9.12	1918	Influenza.

In the twenty-three cases still alive, noted in Table I, the blood was examined by wet and dry films, but in no case were trypanosomes found. It must be noted, however, that Gallagher and Macfie failed to discover trypanosomes in the blood of any of the cases, diagnosis in every instance being made by gland puncture.

Very few glands were punctured during the present enquiry, as in practically all cases the glands had resolved, or there was at most an indefinite general thickening. In a few cases there were one or two very small, extremely hard glands to be felt, fibrosis evidently having taken place. Scarring of the neck to a variable degree was common.

## PART II

Apart from these thirty-five *recorded* cases, I saw at Eket twenty-eight *unrecorded* cases whom I accepted as being former patients at the camp from their history and from evidence, especially from members of the Qua-Ibo Mission, who furnished me with

documentary proof of their personal knowledge of the individuals. These cases were all in good condition, and did not exhibit enlarged lymphatic glands or any other evidence of trypanosomiasis.

I was also informed of the deaths of seven *unrecorded* cases who were stated to have been inmates of the camp.

It is possible that the above-mentioned unrecorded cases are included in Macfie's one hundred and fourteen cases who presented some of the clinical features of the disease, but in whom parasites were not actually found.

### PART III

In endeavouring to ascertain if trypanosomiasis is still prevalent in the Eket District, as many persons as possible were examined in the towns and villages visited. As already stated, great difficulty was experienced, as there appeared to be a very great reluctance to impart information; in fact, so much so, that on several occasions it was necessary to invoke the assistance of the authorities in order to get the people of a particular place to come in for examination. At no place was this more marked than at Ikorubo, the site of the former sleeping sickness camp. I am informed that this was due to the fact that the chiefs and headmen of the various surrounding villages feared being called upon to furnish labour for another and new sleeping sickness camp.

Twenty-three cases were seen which exhibited signs suggestive of sleeping sickness, *e.g.*, enlarged glands, but gland puncture and blood examination were negative.

In all, one thousand eight hundred and six persons have been examined by gland palpation, and the following table shows the result. For purposes of comparison with the results recorded by Macfie and Gallagher (1914), the same age-groups classification of glands has been adhered to:—

- + = Glands obviously enlarged.
- + - = Sufficiently enlarged to be grasped.
- + - - = Enlarged, but not sufficiently to be grasped.
- = Normal.

TABLE III.

The incidence of enlarged posterior cervical glands among 1806 natives classified according to sex and age.

Sex	Male			Female		
Age	0-13	14-44	45-	0-11	12-39	40-
+	5 (0.88%)	4 (0.64%)	0	2 (0.64%)	4 (1.42%)	0
+-	40 (7.04%)	7 (1.13%)	1 (5.0%)	22 (7.1%)	13 (4.63%)	0
+- -	372 (65.5%)	210 (33.87%)	3 (15.0%)	171 (55.16%)	62 (22.06%)	0
-	151 (26.58%)	399 (64.35%)	16 (80.0%)	115 (37.1%)	202 (71.88%)	7 (100%)
Number of individuals of each class examined ...	568	620	20	310	281	7

As regards the above table, it will be seen that a large proportion have glands that can be classified as + - - ; those with + - are considerably smaller, whilst the + is a very small figure to the total. The class -, or normal, equals 49.28 per cent. of the total examined. In plain words, it is very rare to see anyone who has enlarged glands to an extent that is noticeable. Enlarged glands are certainly common, and they usually take the form of small discrete, hard, shotty glands; in many cases they can be very well compared with buckshot. In a few cases the glands were observed to be suppurating, with a well marked sinus, or they were distinctly soft; now and again the post-auricular ones were enlarged, but this was comparatively rare.

Comparison of the above tables with those of Macfie and Gallagher shows a striking reduction in the proportion of individuals with enlarged glands.

The number of 'dirty heads' was most marked, ranging from a simple dry eczema to the most intensely deep punched-out necrotic ulceration. There is no doubt that the 'dirty head' was more common amongst males, and in a large majority of the heads classified 'dirty' the occipital glands were always involved.

Many hundreds of blood slides have been examined, both by the wet and dry methods, but in no case was a trypanosome found; similarly, gland punctures performed whenever possible were invariably negative.

The people of Eket are of a very poor physique, and many of them have the appearance of being ill-nourished and on the verge of starvation. Various diseases—yaws, syphilis, rheumatism—appear to be common. Eket is densely populated, the inhabitants living in scattered houses, and not in any definite towns.

Very few biting flies were found, but a few tsetse were captured.

### SUMMARY

Of the cases seen and referred to in the Annual Medical Report for the year 1913, thirty-five cases have been traced out of a total of eighty-nine recorded by Foran and Gray, and of these twenty-three are alive and in good health (Table I).

In addition, twenty-eight cases have been traced who gave direct information as to their having been in the camp, but whose names were not recorded or cannot be traced. It is possible, however, that some of these twenty-eight cases were amongst the one hundred and fourteen noted by Macfie as presenting some clinical signs of the disease but in whom the condition was not diagnosed microscopically. In this connection it is gratifying to be able to state that the names of the various medical officers who had charge of the camp are well remembered, and on more than one occasion the name of the doctor in charge was given without seeking.

Sleeping sickness has not been demonstrated during the recent tour in Eket, although the cases selected for examination were chosen as presenting clinical signs of possible trypanosome infection. The natives themselves are of the opinion that the disease has died out, and this statement is borne out by the members of the Qua-Ibo Mission; at the same time this must not be accepted as a definite statement, because, as already noted above, the natives are not inclined to discuss the subject, and I am strongly of the opinion that a number of possibly genuine cases were removed from villages on my approach.

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# NOTES ON CULICIDAE IN VENEZUELA, WITH DESCRIPTIONS OF NEW SPECIES

## PART III

BY

A. M. EVANS

(Received for publication 27 February, 1923)

*Anopheles (Arribalzagia) punctimacula*, D. and K.

*Anopheles (Arribalzagia) venezuelae*, Evans

Amongst the *Arribalzagia* material from the Panama Canal Zone, referred to in my previous paper (1922), were twenty-nine specimens with at least one of the hind tarsi complete. Most of these agreed with Howard, Dyer and Knab's (1917) description of *A. punctimacula*, D. and K., but two specimens had two hind tarsal bands as in *A. venezuelae*, and two others showed a tendency to the formation of the second band. Further, a very considerable amount of variation was found among the specimens, with regard to the spotting of the other segments of the tarsi, the left and right hind legs of the same insect in one case being markedly different in this respect. The tarsal characters used by me to distinguish *A. venezuelae* from *A. punctimacula* (1922, p. 217) are, therefore, valueless.

I have also been able to examine numerous other examples of *A. venezuelae*, kindly sent by Dr. Núñez Tovar from Venezuela, among them being seven specimens in which the last hind tarsal segment has only one dark band. It was also found that the most perfect specimens among these collections had a number, from two to fourteen, dark squames scattered throughout the long pale scaled area of the third vein, thus agreeing with the description of *A. punctimacula*, D. and K.

A re-examination of the type of *A. venezuelae* has revealed the fact that one of the wings has several dark scales in this position. I have, therefore, no hesitation in regarding *A. venezuelae*, Evans, as synonymous with *A. punctimacula*, D. and K.

•  
*Culex maracayensis*, n. sp. (fig. 1)

MALE.

*Proboscis* with a narrow whitish band on outer third. *Palpi* with scales mostly dark brown, pale scales creamy, forming a narrow band on basal half and a wide band on proximal half of long segment, bases and apices of all the segments pale scaled. *Occiput* with silvery narrow curved scales in front, brassy ones behind, upright forked scales blackish. *Prothoracic lobes* with whitish narrow curved scales and pale brown bristles. Integument of mesonotum reddish brown. Dorsum with two broad bare stripes, narrowing distally. Vestiture of rather sparsely distributed golden brown and silvery scales, the latter occurring chiefly at anterior lateral margins, on anterior fourth of median area, around anti-scutellar space, and in two small oval areas on posterior half of disc. Bristles, numerous, brown.

*Abdomen.* Tergites dark brown scaled with narrow irregular basal bands of whitish scales. Sternites clothed with transparent whitish scales.

*Wings* with dark brown scales. Bases of fork cells about equidistant from base of wing. First fork cell about twice as long as its petiole.

*Legs.* Femora pale beneath, the pale area being very well defined on the femur. Apices of front and mid femora narrowly pale. Front tibia with conspicuous apical white patch above, about twice as long as the average width of the tibia in dorsal aspect. Mid tibia with very small pale apical spot, pale scaled beneath throughout, hind tibia with a well defined stripe of creamy scales extending along most of its length dorsally and a well defined apical white ring. Front and mid tarsi with first two segments narrowly pale apically, other segments of front tarsi without white, those of mid tarsi with one or two pale scales apically. Hind tarsus, with conspicuous pale rings apically and basally on all the segments.

*Hypopygium* (fig. 1). Side-pieces (A) with clasp narrowing gradually towards distal extremity, articulated spine narrow. Lobe of side-pieces (B) an undivided, distally directed arm, bearing three stout rods, of which two are sub-equal, and longer and stouter than

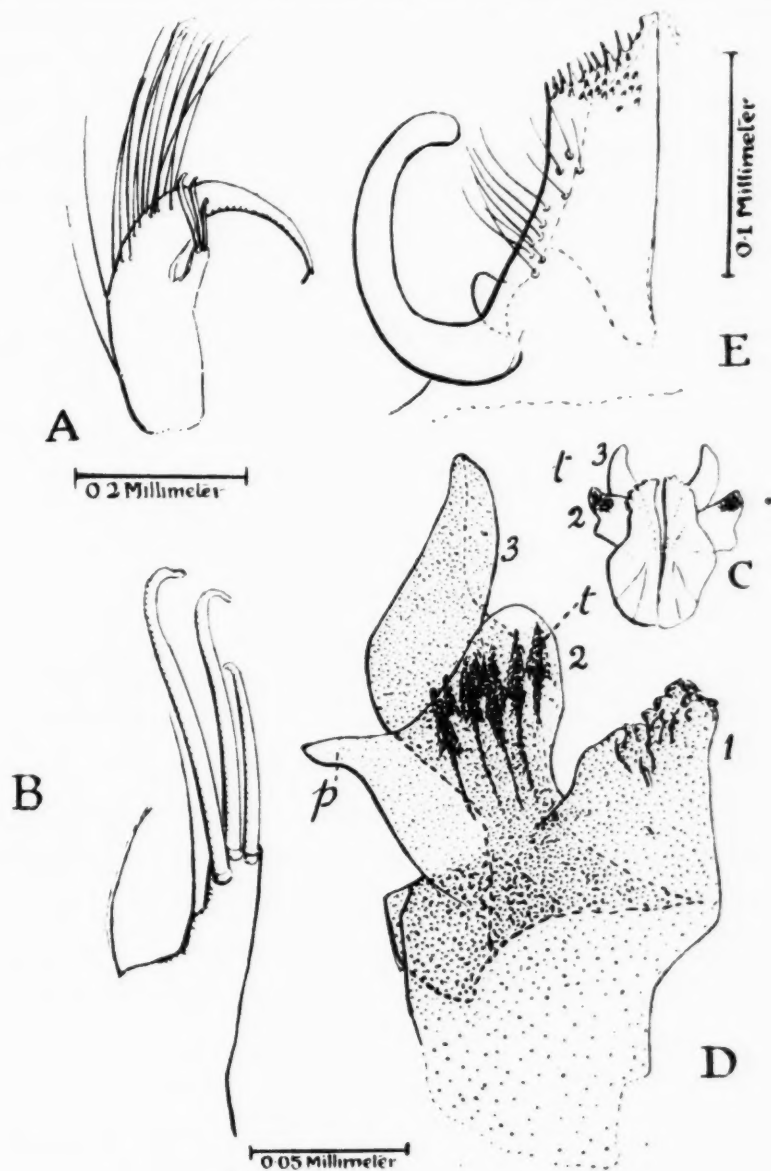


FIG. 1. *Culex maracayensis*, n.sp. Hypopygium. A.—Side piece. B.—Lobe of side piece. C.—Phallosome, ventral aspect, semi-diagrammatic, to same scale as A; 1, 2, 3 lobes numbered as in D.; *t*.—position of teeth. D.—Half of phallosome under pressure, ventro-lateral aspect; *p*.—process of lobe 2; *t*.—teeth on upper side of lobe 2. E.—Tenth segment, dorsal aspect.

the third. *Phallosome* (C & D) with each half divided distally into three lobes; inner lobe (1) ventral, with chitin distally thrown into ridges, which give rise to a denticulate appearance, particularly at the margin. Second lobe when flattened out appearing as a thin

plate with an external pointed process (p.) and bearing on its dorsal surface a row of blackish chitinous teeth, five large and three or more small (some of these teeth are largely obscured by the others in the figure); four of the large teeth continued proximally as thin chitinous ribs. Third lobe (3) arising dorsally to second lobe, elongate, curved and much narrower than the first and second lobes. Tenth segment a membranous lobe with curved basal arms, distal margin spinose, tergal surface with paired chitinous plates, a spinous area distally, and a group of four setae and a row of seven setae laterally.

*Length*, c. 4.0 mm. *Wing*, c. 3.0 mm.

*Type*: One ♂, Maracay, October, 1922; Dr. Núñez Tovar.

This species appears to be most closely related to *C. coronator*, which it resembles in colouration and in the character of the tenth sternites.

*Culex paganus*, n. sp.

MALE.

*Palpi* very short, as short as those of female. *Head*: Antennae plumose, hairs brown; *proboscis* dark brown scaled, expanded apically; *eyes* black, *occiput* black clothed with white, narrow curved scales, white flat ones at sides below, and pale yellowish brown upright forked scales. Clypeus yellowish brown, sub-globular.

*Prothoracic lobes* whitish scaled. Integument of mesonotum pale olivaceous, darker where sub-median bare stripes occur and in posterior lateral areas. Scales whitish and pale yellowish brown, the whitish ones predominating anteriorly and at sides. Bristles long, dark brown. Pleurae pale green.

*Abdomen* with grey integument. Scales of tergites dorsally very dark brown with sub-metallic bluish lustre, ventrally whitish with bluish lustre. Sternites whitish scaled.

*Legs* unbanded, vestiture dark sepia, femora pale beneath.

*Wing*. Scales of costa and sub-costa dark sepia, on other veins semi-transparent with obscure bluish tinge in certain lights. First fork cell almost three times as long as its petiole, second twice as long as its petiole.

*Hypopygium*. The main features are illustrated in figure 2. Tenth sternites slender, comb-shaped distally with about six teeth.



## FEMALE.

Antennae pilose, hairs brown. Occiput with creamy narrow curved scales and pale straw-coloured upright forked ones. Mesonotum with integument uniformly brown, pale scales almost confined to edges of disc and lateral depressed areas.

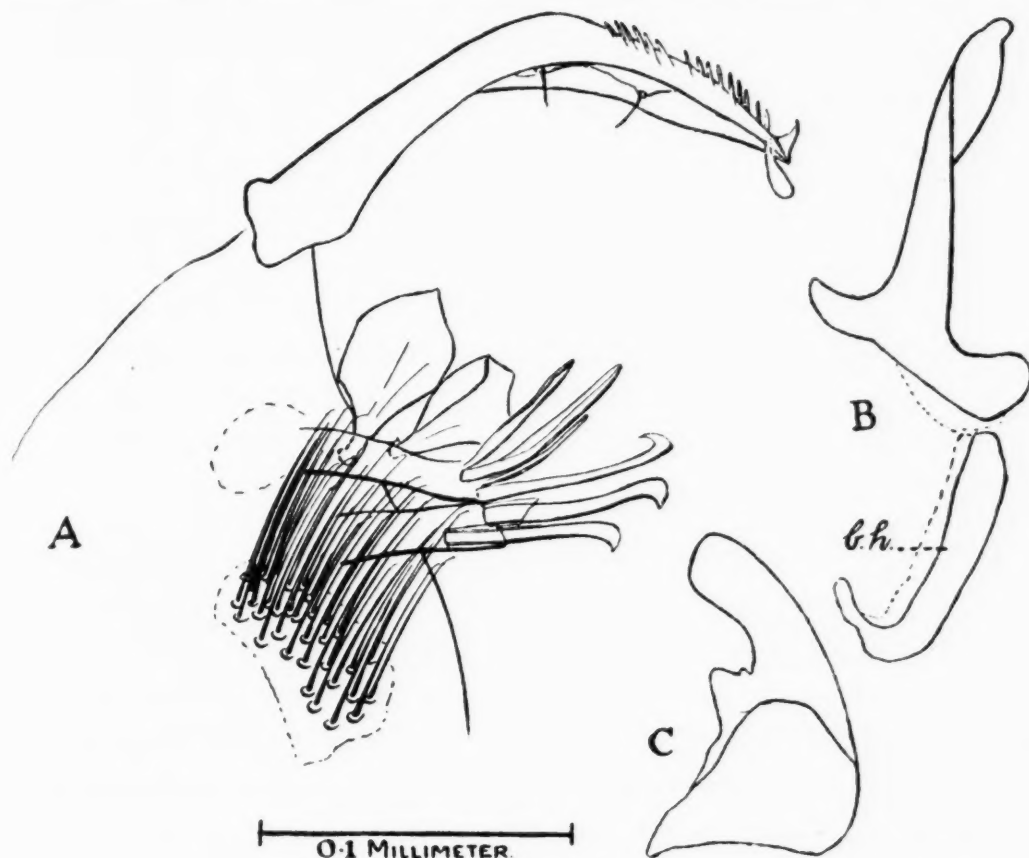


FIG. 2. *Culex paganus*, n.sp. Hypopygium. A.—Apical part of side piece, general setae of vestiture omitted. B.—Half of phallosome, lateral aspect: *b.h.*—basal hooks. C.—Transparent triangular plate.

*Legs.* Hind tibia and metatarsus with a few brassy scales beneath.

Type: ♂ and ♀ from villages, Estada Aragua, Venezuela, 23rd August, 1922; Dr. M. Núñez Tovar.

This species apparently approaches near to *Culex (Isotomyia) bifoliata*, Dyar, from the Panama Canal Zone, in the structure of the male hypopygium. The leaves on the stem of the upper division of the side-piece are, however, described as 'crooked curved leaves,' and although the leaves (*l.*) in *C. paganus* are apt to be folded in mounting, they could not appear crooked unless greatly distorted in this process. There are also a number of other differences in



points of detail, but in the absence of a figure of the structures in *C. bifoliata* it is difficult to estimate the value of these. In vestiture, however, *C. paganus* differs very greatly from the Panama Canal species, in which the upright forked scales of the head are white, the vestiture of the mesonotum consists of 'fine dark brown hairs, and the abdomen is entirely black.' There can be no doubt, therefore, that *C. paganus* is specifically distinct from *C. (Isotomyia) bifoliata*, Dyar.

*Culex (Neomelanoconion) chrysothorax*, Newst. and Thomas

I am now able to confirm the occurrence of this species in Venezuela, which has hitherto rested on the record of a single female collected by Professor Stephens at Mene Grande. Two males and two more females were taken at Maracay, 5th October, 1922, by Dr. M. Núñez Tovar.

*Psorophora tovari*, Evans (figs. 3 and 4)

A considerable amount of material of this species has been received since the publication of its description (1922), which enables me to give a comprehensive account of the thoracic and abdominal colouration, as well as a description of the male.

FEMALE.

*Mesonotum.* The distribution of scales of different shapes and colours is illustrated in figure 3. The narrow curved, spindle-shaped, and smaller broad curved scales (fig. 3, C, D & E), which are usually dull brown or yellowish brown, are in some specimens dull pale yellow and whitish. The very broad, much curved scales (B, B1) are usually pale creamy yellow, sometimes pale yellow.

*Abdomen.* The broad, pale yellow, apical, dorsal bands which are complete on segments two to six of the type, may be interrupted medially by dark scales on segments three to six, four to six, five to six, or six; or they may be separated from the posterior margins medially by a relatively small or large dark scaled triangular patch on these segments.

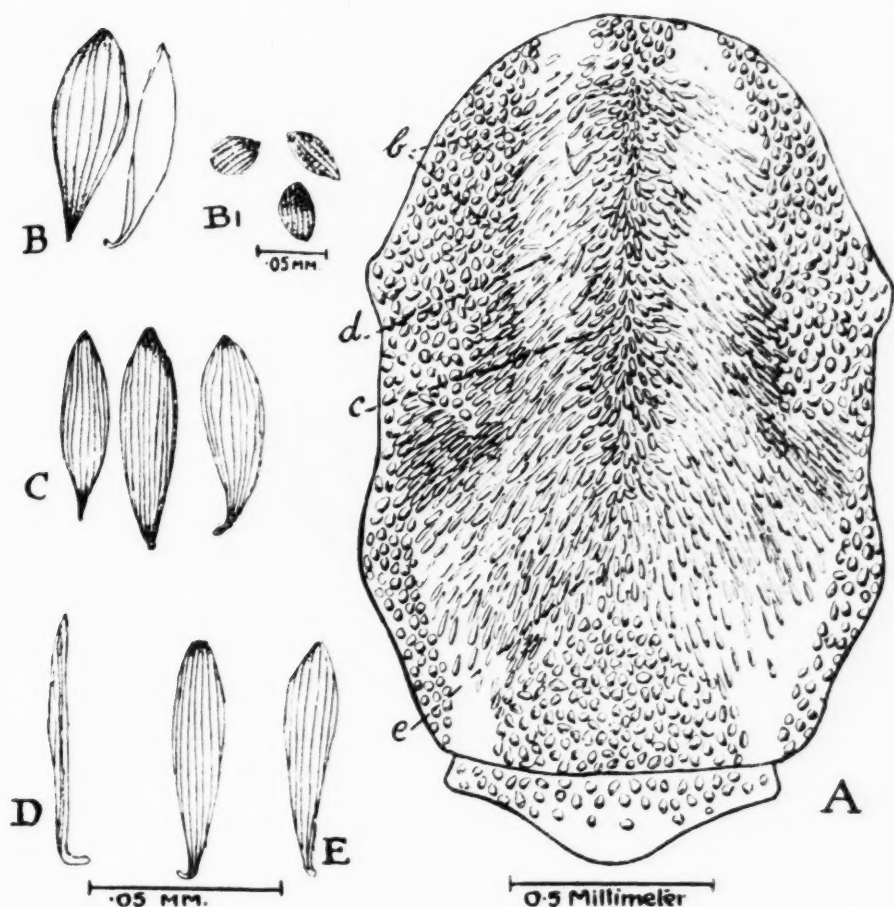


FIG. 3. *Psorophora tovari*, Evans. A.—Mesonotum of female. B., C., D., and E.—Scales from regions b., c., d., and e., of mesonotum, mounted in balsam; B 1.—scales from region b. as seen in situ.

#### MALE

*Palpi* entirely clothed with blackish scales with deep metallic blue reflections and black hairs, last two segments incrassate. Hairs of antennae brown, tori shining black. Occiput, mesonotum, legs and wings as in female.

*Abdomen* with apical bands usually complete on segment two, complete or divided on segment three, and generally interrupted medially either partially or completely on the other segments.

*Hypopygium* (fig. 4). Claspettes (harpagones) with nine (this number may be subject to slight variation) stout filaments (*f.*) arising from prominences along distal border, and a row of about sixteen to twenty very delicate setae with distal portions swollen and produced into fine filamentous processes as shown in the figure.

Type: ♂ and nine co-type ♂♂ from Maracay Region, Venezuela, 1922; Dr. M. Núñez Tovar. Co-type ♀ from Maracay,

10th October, 1922, and others from Maracay Region, July, 1922, ♀♀ 14; Maracay, 5th June, 1922, ♀♀ 30; San Meteo, 2nd June, 1922, ♀♀ 27; Guacara, 1922, ♀♀ 4; Laguna, 15th June, 1922, ♀♀ 2. Dr. M. Núñez Tovar.



FIG. 4. *Psorophora tovari*, Evans. Apical portion of claspette, ventral aspect; *f.*—stout filament; *b.*—expanded hair.

This species is evidently closely allied to *P. cyanescens*, Coq., and *P. purpurascens*, Eds., specimens occurring which resemble one or other of these species in abdominal markings. The three species appear to differ chiefly in mesonotal vestiture; *P. cyanescens* having 'broad soiled silvery scales intermixed with some narrower brown ones . . . especially on centre of disc, but not forming any defined pattern' (H., D. and K., 1915), while *P. purpurascens*, Eds., has the mesonotum with 'flat silvery grey scales, darker, but not conspicuously so, in the centre of the mesonotum.'

#### *Psorophora ciliata*, Fab.

In a previous paper (1922) I recorded the occurrence of two specimens of this species near Maracay, and Dr. Núñez Tovar has subsequently sent further material from this region. In view of

Dyar's recent study of the species of the *ciliata* group of *Psorophora*, and their distribution, and also of the fact that they exhibit considerable differences in thoracic pattern from *P. ciliata*, a further discussion of the Venezuelan specimens is necessary.

Dyar recognises four species of the *ciliata* group of *Psorophora* in the Argentine region, and states that, apart from Theobald's record of it in British Honduras, true *ciliata* has not been recorded south of Tampico, Mexico. Further, he separated *P. tibialis*, a South American species, from *ciliata* by the slight differences of mesonotal pattern together with the markedly discontinuous distribution. Now, in none of the Venezuelan specimens does the mesonotal pattern conform exactly to that of *P. ciliata*, and in some cases (fig. 5, A and B) it differs quite as much as that of *P. tibialis*,

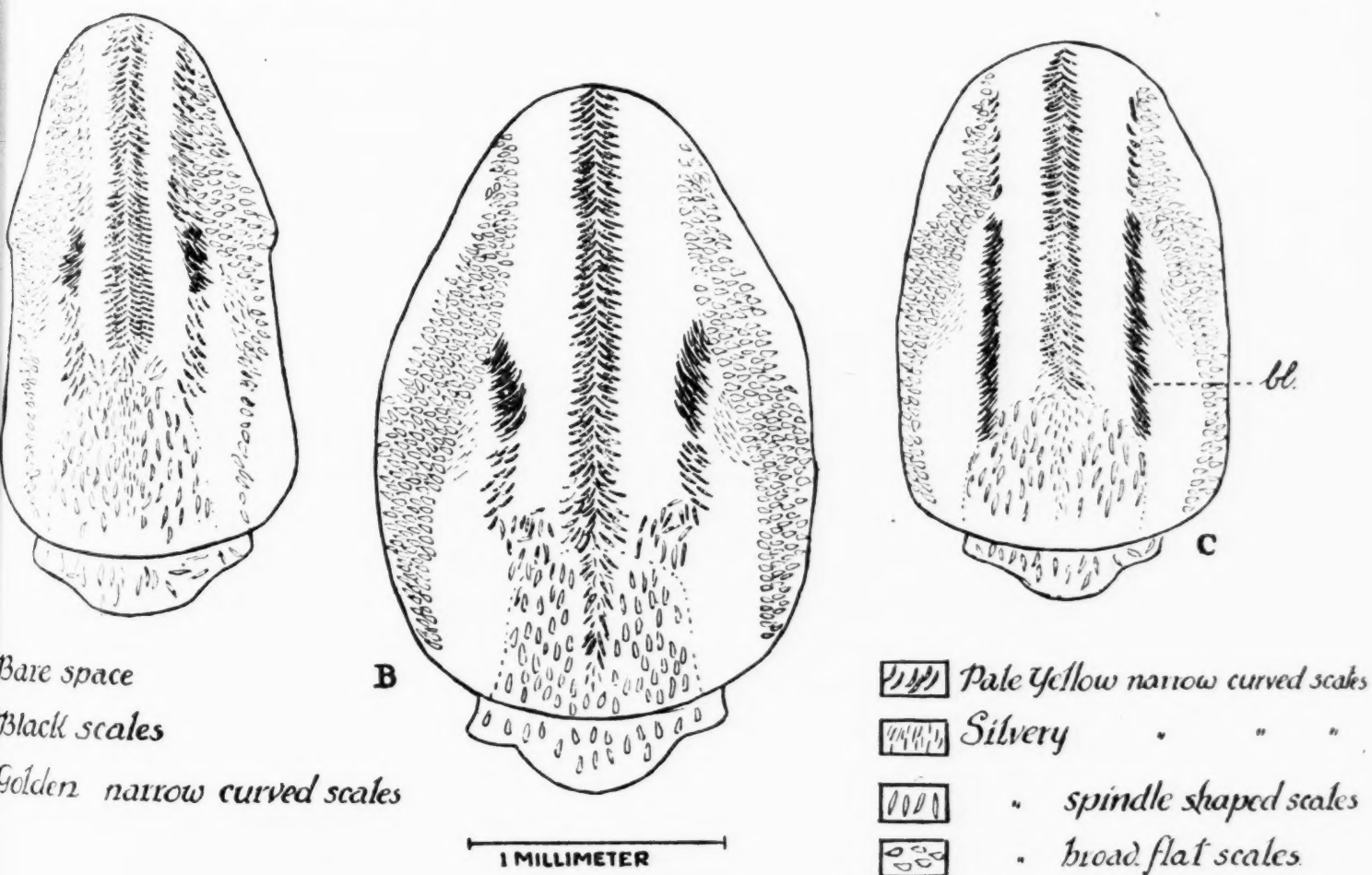


FIG. 5. *Psorophora ciliata* from Venezuela. Mesonotal patterns of three different specimens. A. and B.—Female; C.—Male.



resembling this latter species in the reduction of the long lines of black scales (fig. 5, C, *bl.*). All the five specimens differ from *P. ciliata* in having the median line of narrow curved scales not golden in the antescutellar space. In three of the specimens these scales are entirely silvery (fig. 5, A & C), while in the other two (fig. 5, B) they are mostly pale yellow. Owing to the amount of variation which exists among only five specimens from this region, and the proximity of Venezuela to Central America, I regard these specimens as specifically identical with *P. ciliata*.

*Megarhinus trinidadensis*, D. and K.

Males, larvae and pupae agreeing with the description of this species, and females differing in the absence or reduction of white on the third mid-tarsal segment, have been received from Dr. M. Núñez Tovar. This difference does not seem to justify the separation of these specimens from the Trinidad species.

Bred in laboratory, Maracay, 1st November, 1922, Dr. M. Núñez Tovar, ♂ 1, ♀ 1; Mariara, Est. Aragua, 11th September, 1922, ♂ 1; Maracay, 4th June, 1922, ♂ 1; Maracay region, ♂ 1, ♀ ♀ 20.

*Goeldia longipes*, Fab.

Five females taken at Tucupido, December, 1922; Furmero, 8th June, 1922, ♀ ♀ 2; and Maracay region, June and July, 1922, ♀ ♀ 2, by Dr. M. Núñez Tovar, are referred to this species, although they differ slightly from Howard, Dyar and Knab's (1915) account of it. The mesonotal scales have a distinct sub-metallic blue colour, when the thorax is viewed from behind, and the scales on the scutellum and ante-scutellar space are peacock-blue and greenish-blue. In these respects they resemble *L. culicivora*, D. and K., but they differ from this species, and resemble *G. longipes*, Fab., in the ciliation and colouration of the hind legs. The female palpi are said to be equal in length to six antennal segments in *G. longipes*, and to four in *G. culicivora*; in the Venezuelan specimens the female palpi equal nearly five antennal segments, that portion projecting beyond the clypeus being equal to four segments.



*Wyeomyia (Decamia) pseudopecten*, D. and K.

A male specimen taken at Maracay, 2nd September, 1922, by Dr. M. Núñez Tovar, was found to agree closely with this species in hypopygial characters, but the long paired hairs of the side-piece, though longer than the clasper were less than twice its length.

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*It is with deep regret that we record  
the death of*

*H.R.H. Princess Christian  
Princess of Great Britain and Ireland*

*Honorary President of the Liverpool  
School of Tropical Medicine  
from 1905*



Melana  
Mrs. Christian  
Mrs. J. P. Britton & Ireland  
S



# A REVISION OF THE *AMPHISTOMATA* OF MAMMALS

BY

P. A. MAPLESTONE

(Received for publication 12 January, 1922)

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\* Species in the Museum, Liverpool School of Tropical Medicine.

## INTRODUCTION

The publication by Stiles and Goldberger (1910) of their monograph on the 'PARAMPHISTOMOIDEA' called forth hostile criticism from many writers, notably Braun (1911), Odhner (1911), and Looss (1912). The chief objection of these authors to Stiles and Goldberger's classification is that they ignored the recent attempts that had been made to classify the Trematodes by using affinities in the lymphatic and excretory systems as a basis. Although not agreeing with the classification of Stiles and Goldberger, none of the above writers formulated an alternative system, and although Looss in his article of 1912 says he has been several years trying to group the Amphistomes on the comparative features of the lymphatic, excretory and copulatory systems, this work has not been published yet, so far as the writer is aware. The result is that the confusion caused by Stiles and Goldberger's multiplication of genera, etc., still exists. Stunkard (1917), in a resumé of the group, makes a 'provisional' attempt at reclassification, but as he did not examine any material except three or four species from American fish, for which he makes two new genera, he is unable to discuss whether many of the existing species and genera are valid or not. All that he has done is to remove Stiles and Goldberger's family names, leaving some of their sub-family and their generic names; to add a sub-family of Looss containing one of his own genera, one of Looss', one '(Gen. nov.) *spinulosum*,' and 'Genera of uncertain position'; and to recommend a new sub-family for *Balanorchis* and another sub-family for his own genus *Zygocotyle*. Such a procedure cannot be regarded as improving matters.

It is probable that Looss' suggestion to classify the members of the group on the minute anatomy of the lymphatic, excretory, and copulatory systems is sound, but it appears to be too complicated for practical purposes.

In view of the opportunity presented by the large collection of Amphistomes in the Museum of the Liverpool School of Tropical Medicine, the writer decided to undertake a revision of the group with the object of providing a working classification. This is not based on minute histological study but on easily ascertained anatomical characters, and some of the divisions used by Stiles and Goldberger (e.g., the three families *Gastrodiscidae*, *Paramphistomidae*, and *Gastrothylacidae*) have been retained because they serve to divide the group on easily distinguished external



characters, and are hence of practical use. It is realised that the present attempt at classification does not take cognizance of the standards required by the more advanced systematists such as Looss and Odhner, but it is claimed for this system that it is reasonably simple and consistent, and, so far as the Amphistomes of mammals are concerned, enables a species to be determined with considerable accuracy.

Looss also draws attention to the fact that Stiles and Goldberger failed to make any allowance for variation due to difference in age or the state of contraction of specimens, and have made many species on unsound data in consequence. Throughout the present work these factors have been investigated as fully as possible, and an attempt has been made to show to what extent they influence the appearance of the several species of the group. In their monographs Fischöder and Stiles and Goldberger both deal so fully with the synonymy, that it has not been considered worth while to go back beyond these authors.

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NOTE.—The reader is referred to Cohn (1904), Daday (1907), Looss (1912), and Stunkard (1917), for the most recent information regarding Amphistomes parasitic in hosts other than mammals.

#### AMPHISTOMATA, Rudolphi, 1801, e.p., Nitsch, 1819

*Definition.*—*Digenea*: two suckers, the anterior surrounding the mouth and the posterior terminal or ventro-terminal behind the genitalia; gut forked; excretory pore opening dorsally towards the hinder end; testes generally in front of ovary; almost always thick worms more or less circular in section.

#### KEY TO FAMILIES

- |    |   |     |     |     |     |     |     |                         |
|----|---|-----|-----|-----|-----|-----|-----|-------------------------|
| 1. | Body usually flattened and divided into anterior and posterior portions; ventral pouch absent | ... | ... | ... | ... | ... | ... | <i>Gastrodiscidae</i>   |
|    | Body usually conical and not divided into anterior and posterior portions                     |     |     |     |     |     | 2   |                         |
| 2. | Ventral pouch absent  | ... | ... | ... | ... | ... |     | <i>Paramphistomidae</i> |
|    | Ventral pouch present   | ... | ... | ... | ... | ... |     | <i>Gastrothylacidae</i> |

#### Family PARAMPHISTOMIDAE, Fischöder, 1901.

*Definition.*—*Amphistomata*: body not divided into two portions; ventral pouch absent.

## KEY TO SUB-FAMILIES

- |                                   |                            |
|-----------------------------------|----------------------------|
| 1. Oral diverticula absent ... .. | <i>Paramphistominae</i>    |
| Oral diverticula present ... ..   | 2                          |
| 2. Oral diverticula double ... .. | <i>Cladorchinae</i> *      |
| Oral diverticula single .. ...    | <i>Stephanopharynginae</i> |

Sub-family *PARAMPHISTOMINAE* (Fischöeder, 1901), s.str., Stiles and Goldberger, 1910.

*Definition.*—*Paramphistomidae*, without oral diverticula.

## KEY TO GENERA

- |                               |                       |
|-------------------------------|-----------------------|
| Genital sucker absent ... ..  | <i>Paramphistomum</i> |
| Genital sucker present ... .. | <i>Cotylophoron</i>   |

Genus *Paramphistomum* (Fischöeder, 1901), s.str., Stiles and Goldberger, 1910.

*Definition.*—*Paramphistominae*, without a genital sucker.

Type species *Paramphistomum cervi* (Zeder, 1790), Fischöeder, 1901.

## KEY TO SPECIES

- |  |                         |
|--|-------------------------|
| A. Testes in tandem† ... ..  | 1                       |
| B. Testes diagonal† ... ..   | 6                       |
| 1. Testes lobed ... ..   | 2                       |
| Testes not lobed ... ..  | 5                       |
| 2. Testes with two lobes ... ..                                    | <i>P. gigantocotyle</i> |
| Testes with more than two lobes ... ..                             | 3                       |
| 3. Anterior sucker deeply retracted ... ..                         | <i>P. pisum</i>         |
| Anterior sucker not retracted ... ..                               | 4                       |
| 4. Laurer's canal opens posterior to excretory pore ... ..         | <i>P. cervi</i>         |
| Laurer's canal opens anterior to excretory pore ... ..             | <i>P. orthocoelium</i>  |
| 5. Laurer's canal opens posterior to excretory pore ... ..         | <i>P. liorchis</i>      |
| Laurer's canal opens anterior to excretory pore ... ..             | <i>P. wagandi</i>       |
| 6. Testes lobed, Laurer's canal posterior to excretory pore ... .. | <i>P. explanatum</i>    |
| Testes not lobed, Laurer's canal anterior to excretory pore ... .. | <i>P. buxifrons</i>     |

\* Cohn (1904) created a new sub-family *Diplodiscinae*, all the members of which are found in Amphibians and Reptiles. This sub-family has two oral diverticula and has no constant characters by which it may be distinguished from *Cladorchinae*.

† Refers to mature worms, because in young specimens of *P. cervi*, the testes are sometimes found to be slightly diagonally arranged; by this it is meant that the testes are only slightly out of the mid-line, so that the antero-posterior axis of the worm always intersects both testes, but one testis always lies so far in front of the other that a transverse line can be drawn between them without touching either. In young specimens of *P. explanatum*, the testes lie one on each side of the mid-line, so that the antero-posterior axis passes between them without touching either, but one testis is only slightly in front of the other, so that a transverse line intersects both testes. In mature *P. cervi* the slightly diagonal arrangement is not seen, and the testes appear in tandem, whereas in mature *P. explanatum* the testes are definitely diagonal.

*Paramphistomum cervi* (Zeder, 1790), Fiscoeder, 1901.

## SYNONYMY:—

- Paramphistomum gracile*, Fiscoeder, 1901.  
*Paramphistomum bothriophoron* (Braun, 1892), Fiscoeder, 1901.  
*Paramphistomum microbothrium*, Fiscoeder, 1901.  
*Paramphistomum bathycotyle*, Fiscoeder, 1901.  
*Paramphistomum epiclithum*, Fiscoeder, 1904.  
*Paramphistomum papillosum*, Stiles and Goldberger, 1910.  
*Paramphistomum papilligerum*, Stiles and Goldberger, 1910.  
*Paramphistomum indicum*, Stiles and Goldberger, 1910.

The following is a list of the material available:—

1. Ten bottles from the stomach of bullocks killed in the military slaughter-house at Sierra Leone, West Africa.
2. Two bottles from the stomach of bullocks killed at Khartoum, Sudan.
3. One bottle from the stomach of a bullock killed at Yola, Northern Nigeria.
4. One bottle from the stomach of a bullock killed at Zomba, Nyasaland.
5. One bottle from the stomach of a bullock killed at Blantyre, Nyasaland.
6. One bottle from the stomach of a bullock killed at Accra, Gold Coast.
7. One bottle from the stomach of a bullock killed at Nairobi, Kenya Colony.
8. One bottle from the stomach of an antelope (*Cobus* sp.) shot in the Northern Territory of the Gold Coast.
9. One bottle from the stomach of a Roan shot in Nyasaland.
10. One bottle from the stomach of a bullock in England.
11. Two bottles, host and locality?

Many of the above collections comprised some hundreds of specimens, and other worms of the same group occurred along with them in several instances.

## EXTERNAL ANATOMY

*Size and shape.* The size and shape exhibit such infinite variation as to be of little diagnostic value (Plate V).

*Cuticular papillae.* Fiscoeder (1903) states that in some cases he found a number of small papillae of varying size on the cuticle surrounding the oral opening of *P. cervi*, and he does not agree with von Blumberg (1871) that these papillae are always present. The present investigation is in accordance with Fiscoeder's view, as they were found in some specimens and not in others.

Other points usually discussed under External Anatomy will be included under Internal Anatomy, because their full consideration involves details that can only be made out in sections or in cleared specimens.

## INTERNAL ANATOMY

*Muscular system.* The points requiring special mention in this system will be referred to under special organs.

*Nervous system.* This system was not investigated as it has not been used in specific diagnosis.

*Excretory system.* Fiscoeder (1903) describes and figures the excretory bladder of *P. cervi* as being of a definite shape, with thin smooth walls and with the excretory pore opening well in front of the bladder. Examination of a large number of sectioned specimens leads one to the conclusion that a definite form cannot be assigned to the excretory bladder, which varies according to its degree of distension. The position of the excretory pore in relation to the bladder is also found to vary with the age of the individual; in those with no eggs in the uterus, the pore opens dorsally or dorso-posteriorly of the bladder, and as the uterus gradually fills with eggs the pore appears to pass further and further forward until it is seen to open well in front of the bladder. This point is fully discussed under *P. explanatum*, and as *P. cervi* is found to vary in exactly the same manner no further reference to it will be made here.

*Anterior sucker.* The anterior sucker may be longer than broad, circular, or even broader than long. When contracted the central canal of the sucker may be narrow; on the other hand if its external aperture is widely open, the canal may be funnel-shaped, becoming narrower towards its junction with the oesophagus. The internal surface of the oral cavity may, or may not, be furnished with small papillae, but these vary in exactly the same way as in the case of the cuticular papillae; in fact, owing to their irregular distribution, papillae may be present in some sections and absent in others of the same worm. Fiscoeder (1903) states that he found papillae in all the sectioned specimens he examined.

*Oesophagus.* No special mention of the muscle wall of the oesophagus of *P. cervi* is made by Fiscoeder (1903), but in describing other species, e.g., *P. dicranocoelium* and *P. cotylophorum*, he says that the oesophagus of these two worms is characteristic, because the muscle wall is thicker near the gut fork than near its anterior end; therefore the inference is that the oesophagus of *P. cervi* does not exhibit this thickening. Careful examination of five specimens of *P. cervi* cut in sagittal section gave the following results.



TABLE I.

Thickness of the muscle wall of the oesophagus of *P. cervi*.

							Near its anterior end	Near the gut fork
Specimen 1	...	...	...	...	...	...	32 $\mu$	60 $\mu$
Specimen 2	...	...	...	...	...	...	32 $\mu$	40 $\mu$
Specimen 3	...	...	...	...	...	...	20 $\mu$	40 $\mu$
Specimen 4	...	...	...	...	...	...	16 $\mu$	36 $\mu$
Specimen 5	...	...	...	...	...	...	16 $\mu$	32 $\mu$

In specimens 1 and 2, the oesophagus was shorter than in the other three. From the above it is clear that there is a gradual thickening of the muscle wall of the oesophagus in *P. cervi*, but it is not so marked as in the case of the other two species quoted above, and therefore it is not visible in whole specimens in *P. cervi*. Indeed, even in sectioned specimens it is difficult to appreciate with a low power, because the thickening is very gradual and not very marked, and it is only when the point is specially looked for with a high power that one realises its existence. In the collection of material from the *Cobus* sp. of antelope in the above list, some specimens were found which showed the oesophagus to be slightly bulbous even in whole worms, and on sectioning one of these the muscle wall of the oesophagus was found to be 20 $\mu$  thick at the anterior and 72 $\mu$  thick at its posterior end. Although the difference in this case is considerably greater than among the above specimens, the writer does not consider it sufficiently marked to be taken as a specific difference, and merely records it as a probable variant of *P. cervi*.

*The intestine.* Both the degree of convolution and the precise point of termination of the gut caeca are regarded by Fiscoeder (1903) as important points in differentiating between species closely allied to *P. cervi*. These two points were examined in about 150 specimens cleared in carbolic and in about 40 sectioned specimens. Fifty specimens were taken from one bottle, which were chosen on account of their close similarity in external appearance, as it was thought by this means that variation due to artificial influences could be almost entirely eliminated. The result of the examination of these fifty specimens showed that the caeca were nearly straight in some cases and in others were distinctly convoluted, and



between these two extremes there were others with all degrees of convolution of the gut, which indicates that a variable species is being dealt with. This view was further supported by noting a different amount of convolution of the two caeca in the same worm in several instances. The point of termination of the two caeca also varied considerably; in some they ended quite clear of the anterior border of the posterior sucker, and in others they extended as far as the middle of the posterior sucker. In collection No. 10 of the above list, the worms were more fully extended than in any other collection, and in one of these specimens the gut was found to end 1.6 mm. in front of the anterior border of the posterior sucker. All gradations between the two extremes were seen, and in a few worms the caeca ended at different levels on the two sides. The remaining specimens which were not so uniform in size and shape were found to vary in the same way. It is also stated that the gut caeca terminate in a dorsally directed blind end. This was found to be so in most cases, but three specimens were seen in which the terminal part of the caeca ran ventrally. It is considered, as a result of these findings, that small variations in the gut caeca are not reliable points on which to separate species.

*Posterior sucker.* The ratio of the diameter of the posterior sucker to the length of the worm is used in many instances as a distinguishing feature. For the purpose of examining this character eighty-nine worms from one bottle were measured; all of the specimens used were fixed in a well-extended condition, differences in length from artificial causes such as shrinking being thus practically eliminated. In these eighty-nine specimens the ratio of the diameter of the posterior sucker to the length of worm varied greatly, all intermediate figures between 1:8 and 1:3.5 being found, and the variations were so gradual that it was found impossible to draw a dividing line at any point in the series. A large number of worms from other bottles were also measured in the same way, and as these were in different degrees of contraction the variation was found to be even greater than in the above series; in one strongly contracted example the ratio of sucker to length of worm was only 1:2.5. The collection obtained from England (No. 10) consisted of twelve specimens, which were part of the collection mentioned by Pillers (1922). They were all fully extended and presented a very uniform appearance externally, both in size and shape. Five of these specimens were cleared in carbolie and it was found that the diameter of the posterior sucker in proportion

to the length of the worm in the five specimens was as follows: 1 : 9.5, 1 : 7, 1 : 5.7, 1 : 5.4, 1 : 4.75. All of these specimens were gravid, and as they only varied from about 11 mm. to 10 mm. in length, it is obvious that the size of the sucker varies quite apart from the age and size of the worms. According to Pillers these worms are very uncommon in England, so it is improbable that more than one species is present in this small collection. All of these worms were cleared in carbolic acid, so that the general anatomical details were sufficiently clear to render it certain that only one species was being dealt with, and it was noted that the other characters used as distinguishing features between species of this group did not always occur in worms with a corresponding size of sucker. For instance, a worm with a sucker only one-eighth the length of the worm, and hence belonging to *P. gracile* on this account, might have its other diagnostic points more closely allied to *P. cervi* or any other of the species to be discussed below. This mixture of characters of more than one type could be indefinitely extended until the species became inextricably confused. It is considered, on account of the above observations, that the ratio of the posterior sucker to the length of the worm is much too variable to be used as a diagnostic character.

*Genitalia. Testes.* The size and shape of these organs and their relations to one another and to other structures were found to vary somewhat, depending on differences in age and on the degree of contraction in which the worms happened to be when fixed. One testis always lies behind the other, in or near the mid-line; as a rule one testis is directly behind the other, but in young specimens with small testes they are sometimes slightly diagonally placed. When the testes have grown, however, this diagonal arrangement is no longer so obvious. Also in young worms, and even in fully extended examples with eggs in the uterus, there is sometimes a distinct interval between the two testes up to as much as 1 mm. In these instances the testes are circular in outline. On taking a series of worms of gradually increasing age and degree of contraction, the testes are found to come closer and closer together until they touch; after this they tend to become flattened with consequent extension laterally and dorso-ventrally, so that they appear oblong in shape and their borders approach nearer the periphery; in extreme cases they even cause a bulging of the external surface of the worm. The relation of the posterior testis to the posterior sucker also varies considerably; in young worms and well-extended adults the hinder testis lies altogether in front of the posterior

sucker, but in worms not so well-extended the posterior border of the hinder testis reaches, or overlaps, the anterior border of the sucker. The testes are always divided into lobes, which are more distinct in young worms than in older ones. These conclusions are based on the examination of over forty specimens.

Another point of some interest was that three or four specimens were found with the uterus quite full of eggs and the testes small and indefinite. From this it seems possible that the testes atrophy after fulfilling their functions.

*Vas deferens.* Fiscoeder (1903) made an arbitrary division of this organ into three principal parts dependent on the anatomical characters of each part, and as this division is very useful for purposes of description his nomenclature will be followed in the present paper.

The three portions of the vas deferens are as follows:—

1. *Vesicula seminalis.* = A thin-walled coiled tube formed by the junction of the two vasa efferentia.
2. *Pars muscosa.* = The continuation of the vesicula seminalis, furnished with a fairly thick muscular wall.
3. *Pars prostatica.* = A shorter portion of the tube surrounded by a collection of large cells, the prostatic cells.

The pars prostatica leads into a duct known as the Ductus Ejaculatorius, which unites with the termination of the uterus within the genital papilla, and which is known as the Ductus Hermaphroditicus.

The vesicula seminalis varies greatly in size and amount of convolution in different specimens. These variations depend on several factors, such as the degree of contraction of the worm, and whether the vesicula is empty, partly filled, or fully packed with spermatozoa when the worm is killed. The pars muscosa is liable to vary from the same causes, and to these must be added variation due to the state of contraction of its own muscular wall. The muscle wall of the pars muscosa is composed of two layers, an outer longitudinal layer, and an inner circular layer, so that contraction or relaxation of one or both of these layers can cause considerable variation in the length, diameter, amount of convolution of the duct, or the proportionate thickness of the two muscle layers. Both the vesicula seminalis and the pars muscosa coil freely on themselves and on each other, so that their relations with each other vary greatly in different specimens, and even in different sections of the same specimen. From examination of a large number of sectioned specimens,

the writer has come to the conclusion that the relations of the vesicula seminalis and the pars musculosa are so variable that it is not possible to assign any special type of relation between these two parts to any one species as Fiscoeder does in several instances. The pars prostatica varies considerably in size and shape; this fact is clearly brought out in Table II, which is compiled from the measurement of fifteen individuals cut in sagittal section and mounted serially; the maximum measurements are given in every case.

TABLE II.

The length and breadth in microns of the pars prostatica of fifteen specimens of *P. cervi*.

	Length	Breadth		Length	Breadth
1	178	178	9	436	257
2	218	138	10	475	297
3	218	218	11	535	396
4	257	198	12	594	317
5	277	198	13	594	396
6	297	178	14	594	475
7	317	138	15	594	495
8	317	218			

This table shows a regular gradation of sizes from the smallest to the largest, and from this it is concluded that it is not possible to separate species on this character.

*Genital pore.* The genital pore usually lies about opposite the gut fork or a little behind it, the variation in position depending on the length and course of the oesophagus, which naturally has a direct effect on the position of the gut fork. But in one sectioned specimen which was fully grown and very much contracted, and in consequence only about 4 mm. in length, the genital pore was only  $1/4.8$  of the body length from the anterior end of the worm, and it lay opposite the junction of the middle and posterior thirds of the anterior sucker, that is, far in front of the gut fork. This is not in agreement with the statement of Fiscoeder (1903) who, in his description of *P. cervi*, says:

‘Die Geschlechtsöffnung liegt am hinten Ende des vordern Körperdrittels in der Höhe oder kurz hinter aber niemals vor der Gabelstelle der Darmschenkel.’



*Genital atrium and genital papilla.* These two structures are used extensively in specific diagnosis both by Fiscoeder (1903) and by Stiles and Goldberger (1910). The papilla is composed almost wholly of muscle, and the atrium is surrounded by a special thickening of the subcuticular layer. Accordingly, both are liable to great alterations in form, and further, the papilla is capable of being withdrawn deeply into the body of the worm with resultant deepening of the genital atrium, or else it can be completely extruded through the genital pore, in which case the genital atrium disappears. As would be expected, many worms show intermediate stages between these two extremes. The variations of the genital apparatus are fully discussed in *P. explanatum* (see fig. 2), and as exactly the same type of variations are found in *P. cervi* the reader is referred to *P. explanatum*. The conclusions to be drawn from these facts are that the presence of one or two chambers in the genital atrium, or even the total absence of this cavity, or the size and shape of the genital papilla, are purely a matter of chance and are of no use in distinguishing various species. If Fiscoeder's figures (1903) of *P. cervi* are referred to, it will be noted that in fig. 1 the pore is shown as a small cavity with no sign of the papilla protruding, whereas figs. 2 and 3 show the papilla protruded through the pore and there is no sign of an atrium at all. This suggests that Fiscoeder recognised the possibility of variation in these structures without referring to it in the text. Small papillae are also described as being found on the internal surface of the genital atrium in some species and not in others. Examination of a number of sectioned specimens has shown that these papillae may be present or absent when the atrium is present, and a further fact which makes these structures still more liable to variation is that often the atrium itself is not present.

*Ovary.* It is recognised by all observers that the ovary may vary considerably in position in the same species. The present investigation fully bears this out.

*Shell gland.* This gland always lies close to the ovary, and so varies in company with it.

*Vitellaria.* The vitellaria appear to be the most variable of all the organs in *P. cervi*, and the number of gland groups, their size, and their distribution, undoubtedly increase considerably as age advances. But even when comparing them in over one hundred specimens in which the uterus was well filled with eggs they were found to show marked variation. Among these hundred odd specimens the anterior limits of the vitelline



glands were found to lie as far forward as the hinder end of the anterior sucker in some cases, whereas in others they did not reach as far as the genital pore, and between these two extremes all degrees were found. Sometimes in the same worm the glands did not reach the same level on both sides. The posterior limits of the vitellaria were also found to vary considerably, but as a general rule they ended a little behind the termination of the gut caeca; but this was not invariably the case, for in a few instances the vitelline follicle groups were found extending to the extreme posterior end of the worm and could be made out in the parenchyma surrounding the opening of the posterior sucker. The degree of extension of the vitellaria inwards on the dorsal and ventral surfaces also varied markedly. In some cases the glands were strictly limited to the area outside the intestines on each side, and from this limited distribution all stages of inward extension were found up to a point where the glands of the two sides were practically continuous with one another all along both surfaces. It was noted, however, that when the glands were widely spread they seemed to be sparsely distributed, and when fairly circumscribed in their distribution the groups of follicles were much more closely gathered together. Fiscoeder (1903) uses slight differences in distribution of the vitelline glands for specific diagnosis; the writer does not consider this possible, because in his series of over one hundred gravid specimens of *P. cervi* a much greater range of variation was found than Fiscoeder describes in his different species, and between the extreme limits of these variations all possible degrees were found which made it impossible to separate one species from another.

*Uterus.* The dorsal antero-posteriorly directed portion of the uterus is described as being more convoluted in some species than in others. After a large number of specimens were examined it was realised that the amount of convolution the uterus shows is subject to a wide range of variation in *P. cervi*. This variation was found to be dependent on two factors, first, the amount of contraction of the worm, and second, the number of eggs in the uterus. It was found that in specimens with no eggs the uterus was nearly straight, but as it became more and more filled with eggs the degree of convolution of the uterus increased also. It is therefore considered that slight differences in the amount of convolution of the uterus cannot be taken into consideration in specific diagnosis.

*Eggs.* Eggs taken from the uterus of several preserved specimens of *P. cervi* were found to vary considerably in size, being from  $114\mu$  in length

TABLE III.

Relations of the ovary, shell gland, Laurer's canal and excretory pore in five specimens of *P. cervi* cut in transverse section.

Specimen	A	B	C	D	E
Relations of ovary ...	To right of mid-line. In same dorso-ventral plane as shell gland. Midway between dorsal and ventral surfaces.	To left of mid-line, well towards ventral surface.	In mid-line ventro-posterior to shell gland. Slightly ventral of mid-transverse plane.	Well to right of mid-line in mid-transverse plane.	To left of mid-line: just ventral of mid-transverse plane. Just anterior to hinder border of testis.
Relations of shell gland...	In mid-line. Midway between dorsal and ventral surfaces.	Directly dorsal of ovary.	Just to right of mid-line and slightly dorsal of mid-transverse plane.	Just to right of mid-line antero-internal to ovary and just dorsal of mid-transverse plane.	Immediately dorsal of ovary.
Course of Laurer's canal ...	First dorsally to right of excretory bladder, then turns mesially and crosses to left side anterior to excretory bladder and posterior to excretory duct.	Dorsally and anteriorly on left side of excretory bladder	Dorsally and posteriorly on right side of excretory bladder.	Dorsally and anteriorly on right side of excretory bladder.	Dorsally and posteriorly on left side of excretory bladder
Opening of Laurer's canal ...	100 $\mu$ posterior to and 300 $\mu$ to left of excretory pore. 50 $\mu$ anterior to anterior border of shell gland. 250 $\mu$ anterior to base of ventral sucker.	75 $\mu$ posterior and 400 $\mu$ to the left of excretory pore. 500 $\mu$ anterior to anterior border of shell gland. 325 $\mu$ anterior to posterior border of hinder testis. 650 $\mu$ anterior to base of ventral sucker.	500 $\mu$ posterior and 625 $\mu$ to right of excretory pore. 75 $\mu$ posterior to posterior border of shell gland. On a level with base of ventral sucker.	200 $\mu$ posterior and 300 $\mu$ to right of excretory pore. Directly above shell gland. 175 $\mu$ anterior to base of ventral sucker.	500 $\mu$ posterior and 650 $\mu$ to the left of excretory pore. 100 $\mu$ posterior to posterior border of shell gland. On a level with base of ventral sucker.
Opening of Excretory pore ...	In mid-line 250 $\mu$ anterior to anterior limit of excretory bladder, and 100 $\mu$ posterior to posterior border of hinder testis.	In mid-line, 275 $\mu$ anterior to anterior limit of excretory bladder. 400 $\mu$ anterior to posterior border of hinder testis.	Slightly to left of mid-line, and vertically above left side of excretory bladder. 75 $\mu$ posterior to anterior limit of excretory bladder. On a level with posterior border of testis.	In mid-line 100 $\mu$ posterior to anterior limit of excretory bladder. 75 $\mu$ posterior to posterior border of hinder testis.	Slightly to right of mid-line and vertically above right side of excretory bladder. 125 $\mu$ posterior to anterior limit of excretory bladder. 375 $\mu$ anterior to posterior border of hinder testis.

by  $60\mu$  in breadth up to  $176\mu$  in length by  $90\mu$  in breadth. The above figures represent the extremes of size found after several specimens had been dissected; the maximum variation in eggs from a single worm was only about  $10\mu$  in length and  $7\mu$  in breadth. It is considered on this account that small differences in size of the eggs are not reliable characters for specific diagnosis, at all events when the eggs are taken from the uterus of preserved specimens. It is possible they may be more uniform in size after being laid.

*Laurer's canal.* The relations between Laurer's canal, the excretory bladder, and excretory pore are used by Fischöder (1903) in distinguishing certain species from one another. He evidently attaches great importance to these relations, because he divides the genus *Paramphistomum* into three groups on these characters, and Stiles and Goldberger (1910) have followed Fischöder in this respect. Fischöder's three groups of the genus are as follows:—

1. Laurer's canal crosses the excretory bladder completely. This means that Laurer's canal opens behind the excretory pore and in the mid-line of the worm.
2. Laurer's canal and the excretory bladder do not cross. This means that Laurer's canal opens in front of the excretory pore.
3. Laurer's canal crosses the excretory bladder incompletely. This means that Laurer's canal opens behind the excretory pore, but to the side of the mid-line, i.e., the same side as that on which the shell gland lies.

On account of the importance attached to these characters, five specimens of *P. cervi* all from one bottle and as nearly as possible similar in external appearance, were cut in transverse sections,  $25\mu$  thick, and mounted serially, so as to test the value of the above statements. The result of this investigation is shown in Table III.

Table III and Fig. 1, which consists of camera lucida drawings of Specimens A, B, and C in Table III, show that the relations of Laurer's canal to the excretory bladder and excretory pore vary considerably, and in some cases the relations of these structures do not come under any of Fischöder's three headings. It is probable that if more worms were examined still other arrangements would be found, but it was considered that the above five specimens sufficiently proved that these relations were unreliable for diagnostic purposes. Although only the above five specimens are included in the table, about thirty others were examined; these were cut in sagittal and coronal sections and the above results were thereby

	(1) <i>P. cervi</i>	(2) <i>P. gracile</i>	(3) <i>P. microbothrium</i>
Length	5-12 mm.	11-15 mm.	8-11 mm.
Shape	Posterior end rounded	Almost cylindrical.	Somewhat more flattened ventrally than <i>P. cervi</i> . Only curved ventrally like <i>P. gracile</i> .
Relation of sucker to length of worm	1 : 4 to 1 : 5	1 : 8	1 : 4 to 1 : 5.
Genital pore	Opposite or behind gut fork, $\frac{1}{3}$ of body length from anterior end.	Well behind gut fork, $\frac{1}{4}$ of body length from anterior end.	Behind gut fork, $\frac{1}{4}$ of body length from anterior end. Has a genital atrium and more definite muscular sphincter.
Vesicula seminalis	Very broad, thin walled, in front of anterior testis.	Round canal in median plane.	Lies dorsal of pars muscosa, in <i>P. cervi</i> and <i>P. gracile</i> where it is behind.
Pars muscosa	0.8 to 1.0 mm. long, walls $18\mu$ to $22\mu$ thick, varying with its degree of fulness. Straight or slightly coiled.	$500\mu$ to $600\mu$ long, walls $18\mu$ to $22\mu$ thick. Moderately straight.	Walls $45\mu$ - $50\mu$ thick: almost entirely consisting of circular muscle with a single outer layer of longitudinal muscle. Strongly coiled.
Pars prostatica	$300\mu$ to $600\mu$ in transverse diameter.	$500\mu$ to $600\mu$ long by $250\mu$ to $350\mu$ broad.	$500\mu$ to $600\mu$ long.
Papillae on anterior end and in 'pharynx'	On external surface and in anterior portion of 'pharynx.'	On external surface and not in 'pharynx.'	Not mentioned.
Termination of gut caeca	Dorsal to sucker.	Anterior to sucker.	Anterior to sucker.
Testes	ANTERIOR: 2 mm. to 2.8 mm. dorso-ventral. 1.5 mm. to 2 mm. transverse. POSTERIOR: 2.8 mm. to 3.5 mm. dorso-ventral. 1 mm. to 1.5 mm. transverse. Slightly on opposite sides of middle line, close to ventral surface.	ANTERIOR: Oval, 1.2 mm. by 0.7 mm. slightly dorsal. POSTERIOR: More rounded, 0.9 mm. by 1.0 mm., slightly ventral. Only lie slightly on opposite sides of median line.	ANTERIOR: 2.3 mm. to 2.5 mm. dorso-ventral. 1.5 mm. to 1.7 mm. transverse and longitudinal. POSTERIOR: As a rule somewhat larger. A little more markedly on opposite sides of mid-line. Close to ventral surface.
Vitellaria	Extend from 'Pharynx' to posterior border of ventral sucker. Extend on dorsal and ventral surfaces. In coarse groups of follicles close together.	Extend from hinder border of 'Pharynx' to anterior border of sucker; do not extend markedly on dorsal and ventral surfaces. In five groups of follicles somewhat irregularly placed.	On one side reach from posterior border of 'Pharynx' to anterior border of sucker. On other side from gut to middle of sucker. Similar to <i>P. cervi</i> in size and extent on dorsal and ventral surfaces.
Ovary	Close behind base of sucker, either to right or left of mid-line.	Behind posterior testis either to left or right of mid-line and slightly towards ventral surface (i.e., anterior to sucker).	Further from mid-line than in <i>P. cervi</i> and <i>P. gracile</i> .
Uterus	Portion dorsal of testes markedly wavy.	Portion dorsal of testes wavy.	As in <i>P. cervi</i> .
Eggs	$145-156\mu \times 75-82\mu$ .	$115-125\mu \times 72-80\mu$ .	$145-150\mu \times 75-80\mu$ .
Laurer's canal	Runs dorsally towards anterior and opens in mid-line about level of posterior border of the hinder testis. 1-1.2 mm. behind excretory pore.	Curves posteriorly and opens at level of anterior border of ovary 1.5 mm. behind excretory pore.	Opens to one side and not in mid-line opposite anterior border of ovary $250-300\mu$ behind excretory pore.
Excretory bladder	Flask-shaped, close to dorsal surface. Pore opens anteriorly in mid-line. Crossed by Laurer's canal at junction of anterior and middle third.	Further from dorsal surface. Pore on a level with hinder border of posterior testis. Is crossed by Laurer's canal about its centre.	Similar to <i>P. cervi</i> . Pore in mid-line. Laurer's canal does not cross bladder.

\* At the first glance *P. botbriophoron* appears to have several rather marked points of difference from the other five species: the pars muscosa is closely coiled; the pars prostatica is very long; and the genital atrium is large and deep, with no genital pore.



1924), as typical of the following six species of *Paramphistomum*.

(4) <i>P. epiclitum</i>	(5) <i>P. bathycotyle</i>	(6) <i>P. bothriophoron</i> *
9 mm.	11-15 mm.	6-9 mm.
Anterior $\frac{1}{4}$ strongly curved ventrally, the remainder straight, as in <i>P. cervi</i> : the greatest circumference at 2nd and 3rd thirds.	Ventrally curved. Greatest transverse diameter near posterior end.	Of 4 specimens: 2 strongly curved; 2 slightly curved ventrally.
3 to 1:45.	In text $\frac{2}{5}$ of body length; in drawing $\frac{1}{3}$ of body length.	Not mentioned.
Junction of 1st and middle thirds. Well behind gut fork.	About middle of anterior third behind gut fork. Very little muscle surrounds it.	In middle of anterior half. Not so well developed as in other species. Like <i>P. microbotrium</i> .
Dorsal and posterior of pars musculosa.	Lies loosely coiled between intestines.	Behind and not dorsal to pars musculosa. Closely coiled.
Walls $18\mu$ to $22\mu$ thick.	Ventral and anterior to vesicula, $0.6-0.75$ mm. long and $18\mu-22\mu$ thick, not coiled.	Strongly developed, not loosely coiled like others, but closely coiled. Muscle different from all other species because, unlike those which have nearly all circular muscle surrounding a single layer of longitudinal, the longitudinal is nearly as thick as the circular.
Unlike <i>P. cervi</i> , which is round, it is long $60\mu-800\mu$ , and $250\mu-300\mu$ thick.	Almost globular, $400\mu-500\mu$ in diameter.	$1.0-1.2$ mm. long.
Not mentioned.	On both external surface and in 'pharynx.'	Not seen.
Side base of sucker.	Close in front of anterior border of sucker.	Close in front of sucker.
ANTERIOR: $1.8-2.2$ mm. dorso-ventral. $0.9-1.2$ mm. longitudinal. $1.2-1.6$ mm. transverse. POSTERIOR: $2.1-2.5$ mm. dorso-ventral. $0.7-0.1$ (? $1.0$ ) mm. longitudinal $1.6-2.0$ mm. transverse.	Longitudinal: $1.0-1.3$ mm. } Both about Dorso-ventral: $1.5-1.8$ mm. } same. Like <i>P. cervi</i> , slightly out of mid-line.	ANTERIOR: $0.7-0.8$ mm. long. POSTERIOR: $0.8-1.0$ mm. long. Both $2.0-2.3$ mm. dorso-ventral. More deeply lobed. Lie in similar position to <i>P. microbotrium</i> .
different sizes and very irregular, but close together. Stretch from beginning of oesophagus as far as base of sucker. Reach further on dorsal and ventral surfaces than in <i>P. cervi</i> .	Almost from 'pharynx' to opposite anterior border of sucker. Almost confined to lateral fields only slightly encroaching on dorsal and ventral surfaces. Single follicles small.	From gut fork almost to middle of sucker and also extend on dorsal and ventral surfaces.
Between posterior testis and base of sucker and on same side of mid-line as anterior testis.	Similar position to <i>P. cervi</i> , but only slightly away from mid-line.	Between hinder testis and sucker very near ventral surface and very much to the side.
in <i>P. cervi</i> .	Broad and filled with eggs.	Filled with eggs.
$15-155\mu \times 75-80\mu$ .	$115-125\mu \times 70-75\mu$ .	$125-135\mu \times 65-70\mu$ .
opens in mid-line about the level of its origin.	Runs directly dorsal and opens on a level with ovary.	Opens opposite side to ovary as in <i>P. microbotrium</i> , opposite anterior border of shell gland.
opens on level of middle of posterior testis $300-600\mu$ anterior to Laurer's canal. Laurer's canal crosses between 1st and 2nd thirds of bladder.	Different from <i>P. cervi</i> because it has a long anteriorly directed canal which opens opposite posterior border of anterior testis about middle of length of worm.	More rounded; in front of sucker near dorsal surface.

longitudinal muscle of the pars musculosa is said to be very thick, and it is figured as being arranged in distinct columns; the is conceivable that all of these conditions could be caused by one factor, viz., contraction of the longitudinal muscle of the pars



confirmed. But one point was found to be of value in regard to the relations of the opening of Laurer's canal and the excretory pore, and that is, that in species in which Laurer's canal is described as opening behind the excretory pore this is invariably the case, although the actual distance at which one pore lies behind the other varies a good deal.

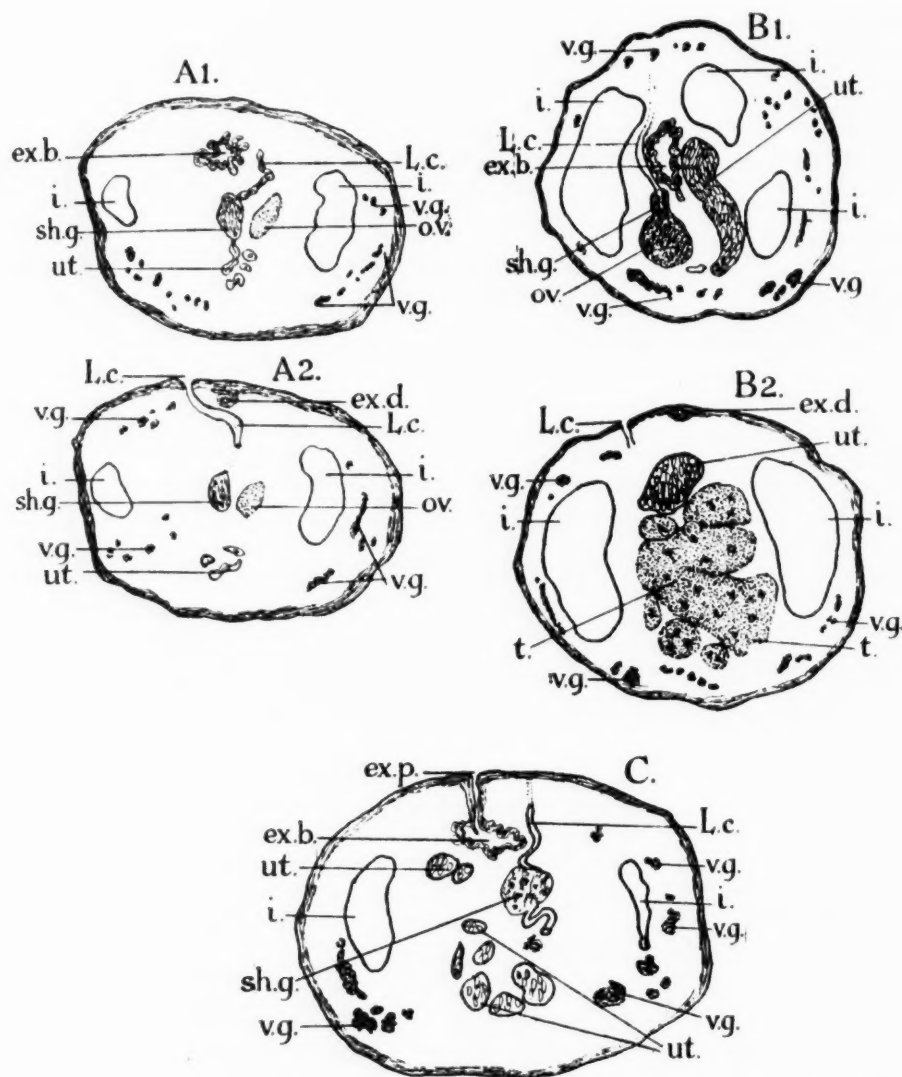


FIG. 1. *Paramphistomum cervi*, transverse sections. A1 and A2—Sections of one specimen at different levels. B1 and B2—Sections of a second specimen at different levels. C—Section of a third specimen passing through the excretory pore. ex.b.—excretory bladder; ex.d.—excretory duct; exp.—excretory pore; i.—intestine; L.c.—Laurer's canal; ov.—ovary; sh.g.—shell gland; ut.—uterus; v.g.—vitelline gland.  $\times 16$ .

Careful examination of the descriptions of the six species of *Paramphistomum* in Table IV shows that the differences between them are very minute, and a further comparison of this table with the results of the extensive examination that has been carried out by the writer shows that with one exception all the characters used by Fiscoeder as of specific

value in distinguishing these six species come within the range of what appear to be variations of *P. cervi*. The exception is the length of the pars prostatica; the maximum length observed by the writer for this organ was  $594\mu$ , whereas Fischöeder gives it as  $800\mu$  in *P. epiclitum*, and 1.2 mm. in *P. bothriophoron*. But this is such a small difference that the author is inclined to disregard it and to consider it probable that the above species are all one.

There are three species, *P. papilligerum*, *P. papillosum* and *P. indicum*, described by Stiles and Goldberger (1910) which also appear to the writer to be identical with *P. cervi*.

*Paramphistomum papilligerum*, Stiles and Goldberger, 1910.

Apparently the material on which this species was made consisted of a series of frontal sections loaned to the authors by Shipley.

The only difference between this species and *P. cervi* is that *P. papilligerum* is described as having small papillae on the inner surface of the genital atrium. It has already been shown that papillae in this position may be present or absent in *P. cervi*, and further that the presence of a genital atrium itself is a variable character. There is hence no justification for the separation of this species.

*Paramphistomum papillosum*, Stiles and Goldberger, 1910.

The material at the disposal of the authors is stated to be a single non-gravid specimen. The species is characterised by the presence of papillae on the anterior end of the worm, in the oral cavity, and in the genital atrium. Papillae in these positions have already been shown to be variable characters of *P. cervi*, being present or absent in one or all of these positions. In *P. papillosum* the excretory pore is stated to be dorsal to the excretory bladder, and in *P. papilligerum* it is stated to be anterior to the bladder; but it must be remembered that in their descriptions of these two worms, Stiles and Goldberger state that eggs were present in the uterus of *P. papilligerum*, and not in *P. papillosum*. It has already been shown that in *P. cervi* the excretory pore appears to pass forward as age advances; this fact is in agreement with the difference observed between the species *P. papilligerum* and *P. papillosum*, when it is remembered that the former is probably a younger specimen than the latter. It is therefore considered that *P. papillosum* is synonymous with *P. cervi*.

*Paramphistomum indicum*, Stiles and Goldberger, 1910.

These worms were said to have been found in two bottles along with other species, and no eggs were observed. The description and drawings of this species by Stiles and Goldberger agree in all points with many specimens of young *P. cervi* without eggs in the uterus, examined by the writer during the course of the present investigation. That the writer's specimens referred to were young *P. cervi* and not another species is rendered practically certain, because in the same bottles worms in all stages of development could be found, so that the gradual change from worms similar in appearance to *P. indicum* could be followed in a long series until a typical *P. cervi* with uterus full of eggs could be found. The writer is therefore of the opinion that on its present description *P. indicum* cannot be distinguished from young *P. cervi*.

In the writer's opinion *P. gracile*, *P. bothriophoron*, *P. microbothrium*, *P. epiclitum*, *P. bathycotyle*, *P. papilligerum*, *P. papillosum* and *P. indicum* are all synonyms of *P. cervi*.

*Paramphistomum liorchis*, Fischöeder, 1901.

This worm is easily distinguished from other species of the genus, because the testes are not lobed; it is the only species in which the testes exhibit this character, and in which Laurer's canal opens behind the excretory pore.

*Paramphistomum pisum*, Leiper, 1910.

The material available for examination consisted of two collections, about thirty specimens in all. The location of the parasite in the host (Hippopotamus) is not given, but Leiper (1910) states that it is found in the small intestine.

No gravid specimens were observed by the writer, but about half the material was sexually mature. Leiper says the worms are pisiform when fresh and somewhat contracted when preserved. This agrees with the writer's observations for all of his specimens were slightly contracted with the result that they were almost globular in shape, the largest measuring about 3mm. in diameter. The worms agreed essentially with Leiper's description; both suckers communicate with the exterior by narrow canals as Leiper figures them, but in three specimens which the writer

sectioned the anterior sucker was found immediately beneath the cuticle as in other members of the genus, and is not separated from it by a canal of considerable length as in Leiper's fig. 34.

*Paramphistomum gigantocotyle*, Brandes, 1896.

Host :—*Hippopotamus amphibius*. Location :—Stomach. Locality :—Africa.

As the original description of this species is somewhat inadequate, Leiper (1910) redescribed it.

According to Leiper this worm may be distinguished from other species by the relative size of the posterior sucker and by the shape of the testes. Although the diameter of the posterior sucker given by Leiper, viz., 3.2 mm. in a worm of 8 mm. in length, is relatively great, it is not by itself a sufficient character for identification, because in *P. explanatum* the relative size of the sucker is often more than this. But the testes appear to be characteristic, as although they are placed one behind the other as in *P. cervi*, they differ in that whilst in the latter they are divided into several lobes, in *P. gigantocotyle* each testis is nearly completely divided into two portions by a deep transverse groove, so that in sections they may appear as four organs, a condition that is never found in *P. cervi*.

*Paramphistomum explanatum* (Creplin, 1847), Fiscoeder, 1901.

SYNONYMY :—

*Paramphistomum calicophorum*, Fiscoeder, 1901.

*Paramphistomum crassum*, Stiles and Goldberger, 1910.

*Paramphistomum cauliorchis*, Stiles and Goldberger, 1910.

*Paramphistomum fraternum*, Stiles and Goldberger, 1910.

*Paramphistomum siamense*, Stiles and Goldberger, 1910.

The material available consisted of the following collections :—

1. One bottle from the stomach of a bullock killed at Durban, South Africa.
2. One bottle from the stomach of a bullock killed at Townsville, Australia.  
(Over 100 specimens.)
3. One bottle from the stomach of a bullock killed at Khartoum, Sudan.
4. One bottle from the stomach of a bullock killed at Blantyre, Nyasaland.
5. Two bottles from the stomachs of two hartebeests shot near Blantyre, Nyasaland. (Both bottles contained over 100 specimens.)

This worm is readily distinguished from *P. cervi*, because the testes are always diagonally situated one overlapping the other, both laterally and antero-posteriorly, in fully grown worms, whereas in full-grown *P. cervi*, the testes lie one directly behind the other.



At the beginning of his description of *P. explanatum*, Fischöeder (1904) states that this species most nearly resembles *P. bathycotyle* on account of its shape, and throughout his paper he contrasts these two species without reference to others. Plate VI, fig. A, clearly indicates that shape cannot be taken as a diagnostic point; the fact that the testes in *P. bathycotyle* are placed antero-posteriorly at once distinguishes it from *P. explanatum* and renders further comparison of these species unnecessary. Apart from the arrangement of the testes the general anatomy of *P. explanatum* is very similar to that of *P. cervi* and the organs are found to be subject to similar variations. The relatively large size of the posterior sucker is apparently regarded as an important point in distinguishing between *P. explanatum* and other species. Fischöeder (1904) states that the worm varies from 8 mm. to 13 mm. in length. But in giving the size of the posterior sucker, he makes use of a single specimen 8 mm. in length and gives no particulars of the dimensions of the suckers in larger worms. The sucker of this single specimen is stated to be 3.5 mm. in its antero-posterior diameter, and 3.0 mm. transversely. That is, the greatest diameter of the sucker to the length of the worm is as 1 : 2.3.

Ten specimens of this worm were cleared in carbolic acid and the length of the worms and the size of the posterior sucker ascertained; these are given in Table V.

TABLE V.

Measurements of ten specimens of *P. explanatum*.

Specimen	Length of worm in mm.	Diameter of sucker in mm.	Ratio of diameter of sucker to length of worm
1	10.1	3.5 × 2.4	1 : 2.9
2	9.7	2.8 × 2.8	1 : 3.6
3	9.6	2.9 × 2.7	1 : 3.3
4	8.5	3.6 × 3.6	1 : 2.4
5	7.6	3.8 × 3.8	1 : 2.0
6	6.8	3.5 × 3.5	1 : 1.9
7	6.8	3.5 × 3.5	1 : 1.9
8	6.6	2.6 × 2.6	1 : 2.5
9	6.6	2.6 × 2.6	1 : 2.5
10	6.0	2.6 × 2.6	1 : 2.3

Average 1 : 2.5



From this table it is clear that the ratio of the diameter of the sucker to the length of the worm varies above and below Fiscoeder's figure, which closely approximates to the mean. On the whole the sucker is relatively larger than in *P. cervi*, but it is subject to such variations that it cannot be taken as an absolute guide in diagnosis. Another point brought out in the above table is that the sucker is not typically oval in shape, being only occasionally met with in this form.

In describing the genital apparatus, Fiscoeder states :

‘Auch das genitalatrium ist nur sehr klein. Die dasselbe umgebende, von dem übrigen Körperparenchym wenig abgegrenzte Musculatum ist nur 0·08-0·1 mm. stark, und die in Grunde des Atriums befindliche Papille ist ebenfalls nur äusserst schwach entwickelt.’

Fig. 2 consists of drawings of the genital apparatus of three specimens of *P. explanatum* cut in sagittal section and from these it is clear that such a precise description as the above of this portion of the worm is not permissible. Although this point has not been figured in detail in dealing with other species, the same range of variation in the genital papilla and genital atrium has been found. Fiscoeder says that the genital organs are displaced towards the anterior end of the worm, and that the testes reach near to the ventral surface, in which case they are of necessity in front of the posterior sucker. In some instances this is correct, as is illustrated in fig. 3, but that the testes are not invariably in this position is shown in figs. 4 and 5. In these two worms the testes are seen drawn away from the ventral surface and they lie dorsal to the posterior sucker. In all probability this is an artificial condition brought about by contraction of the worms at the time of fixation, because both these specimens were noted to be considerably contracted before they were cut, and this is borne out by the irregularity of outline shown in the drawings on the dorso-posterior portion of the worm, especially in the case of fig. 4. This probability is further supported by the appearance in fig. 6, which is a sagittal section of an immature well-extended specimen ; in this drawing the single testis figured is seen to be situated nearer to the ventral than to the dorsal surface and well in front of the sucker. The four worms from which the above drawings were made all came from the same bottle, and specimens in varying degrees of contraction and of different ages are found with characters intermediate between them.

If fig. 6 is examined, it will be readily understood how by contraction of the worm the testes are brought to the position seen in the two previous

figures. The base of the posterior sucker slopes backwards and towards the dorsal surface, and antero-posterior contraction of the worm would cause the testes to impinge on this surface, when they would follow the path of

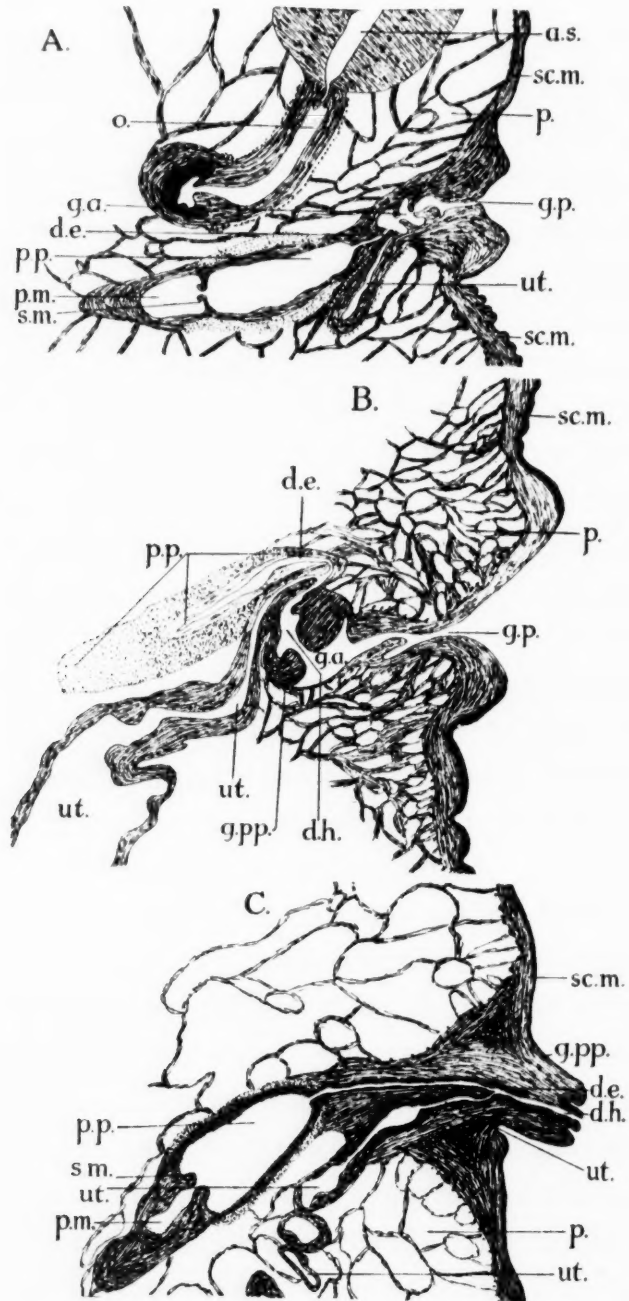


FIG. 2. *Paramphistomum explanatum*. Sagittal section through the genital pore of three specimens. A—Genital papilla fully retracted. B—Genital papilla partly retracted. C—Genital papilla fully extruded. a.s.—anterior sucker; d.e.—ductus ejaculatorius; d.b.—ductus hermaphroditicus; g.a.—genital atrium; g.p.—genital pore; g.pp.—genital papilla; p.—parenchyma; p.m.—pars muscosa; p.p.—pars prostatica; sc.m.—subcuticular muscle; s.m.—sphincter muscle; ut.—uterus.  $\times 30$ .

least resistance and pass up towards the dorsal surface of the worm; at the same time the testes exert some pressure on the sucker, causing its base to

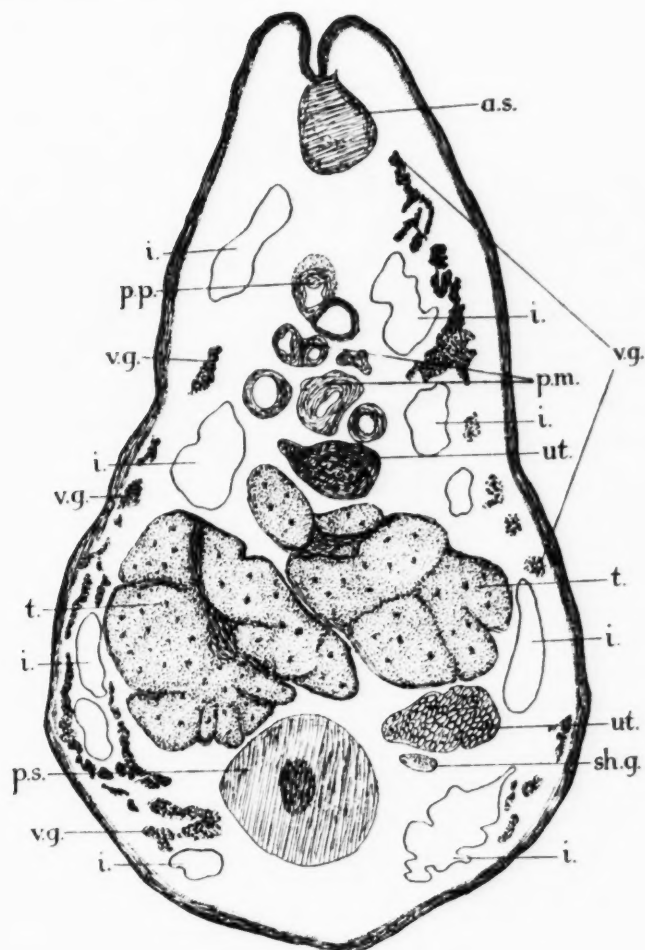


FIG. 3. *Paramphistomum explanatum*. Coronal section of gravid worm. *a.s.*—anterior sucker; *i.*—intestine; *p.m.*—pars muscosa; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t.*—testis; *ut.*—uterus; *v.g.*—vitelline gland.  $\times 12$ .

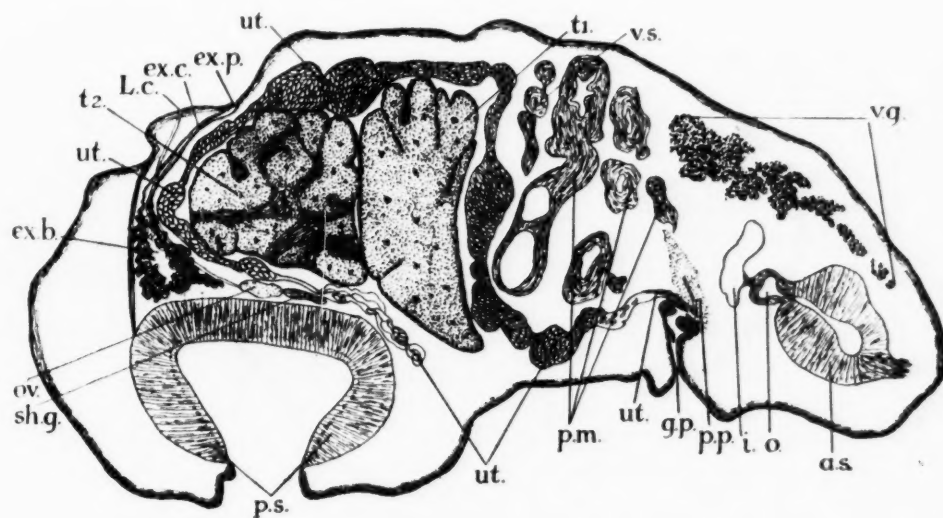


FIG. 4. *Paramphistomum explanatum*. Sagittal section of gravid worm near the mid-line. *a.s.*—anterior sucker; *ex.b.*—excretory bladder; *ex.c.*—excretory canal; *ex.p.*—excretory pore; *g.p.*—genital pore; *i.*—intestine; *L.c.*—Laurer's canal; *o.*—oesophagus; *ov.*—ovary; *p.m.*—pars muscosa; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t1.*—anterior testis; *t2.*—posterior testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.s.*—vesicula seminalis.  $\times$

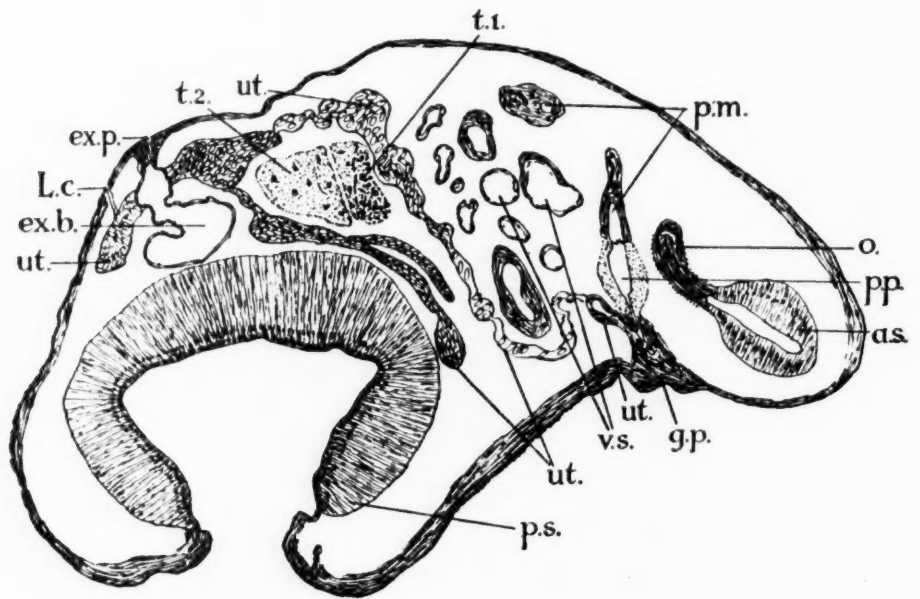


FIG. 5. *Paramphistomum explanatum*. Sagittal section of a partly gravid worm near the mid-line. a.s.—anterior sucker; ex.b.—excretory bladder; exp.—excretory pore; g.p.—genital pore; L.c.—Laurer's canal; o.—oesophagus; p.m.—pars musculosa; p.p.—pars prostatica; p.s.—posterior sucker; t.1.—anterior testis; t.2.—posterior testis; ut.—uterus; v.s.—vesicula seminalis.  $\times 16$ .

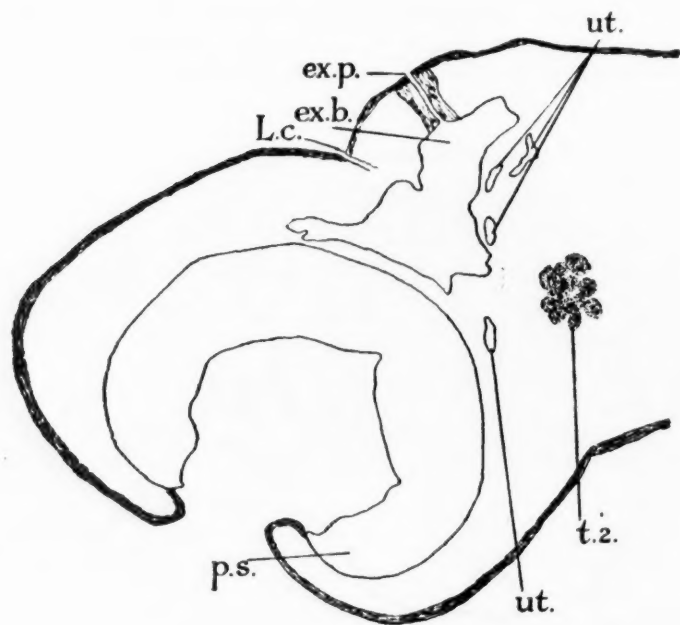


FIG. 6. *Paramphistomum explanatum*. Sagittal section of posterior part of immature worm near the mid-line. ex.b.—excretory bladder; exp.—excretory pore; L.c.—Laurer's canal; p.s.—posterior sucker; t.2.—posterior testis; ut.—uterus.  $\times 16$ .



become flatter and also making it look almost directly ventrally instead of ventro-posteriorly. The full effect of such contraction is shown in fig. 4.

Fischoeder describes and figures the excretory bladder as being of a long flask-shaped type with a narrow canal running forward from it and opening about the level of the posterior border of the hinder testis. Figs. 4, 5 and 6 clearly show that the shape of the excretory bladder is very variable and that the excretory canal runs dorsally from the bladder in young worms, whereas in older specimens it becomes longer and narrower until, in fully gravid worms, it opens well in front of the bladder.

Stiles and Goldberger (1910), in their diagnostic key of the genus, place *P. explanatum* in the group of worms with the 'excretory pore prevesicular,' and in the sub-group in which the following characters are given as diagnostic. 'Testicular fields median, coincide or overlap, zones lobate, testes much smaller than and near the acetabulum; ventral chamber absent; excretory vesicle long and narrow; acetabulum very large.' Their specific definition is: 'Genital pore in postbifurcal zone; musculosa (?); prostatica longer than musculosa; acetabulum less than half as long as body; caeca sinuous, moderately broad, end in acetabular zone; body 8 to 13 mm. long; type host *Bos indicus* at Berlin, Germany.'

With the exception of the relation of the testes to each other all of the above diagnostic characters are so variable that none of them are reliable.

*Paramphistomum calicophorum*, Fischoeder, 1901.

At the outset of his description of this worm Fischoeder (1903) states:

'Das Material ist stark geschrumpft, brüchig; trotz Behandlung mit Iod und Wochen langer Einwirkung von Kreosot lässt sich eine zur Untersuchung von Totalpräparaten nur wenig brauchbare Durchsichtigkeit erzielen, mit Ausnahme von einzelnen unreifen Exemplaren aus dem letzt genannten Glase. Dagegen ist es mir gelungen, von reifen Exemplaren aus dem Glase No. F. 659 für meine Zwecke einiger Maassen brauchbare Schnittserien anzufertigen.'

From this it is obvious that he was unable to obtain a dorso-ventral view of a gravid worm, and, from his figures, he was apparently content with sagittal and transverse sections of full-grown specimens; the only dorso-ventral view he gives is that of an immature worm with the testes small and wide apart, an arrangement which is quite unlike that found in gravid specimens. Thus he failed to realise the identical arrangement of the testes in mature *P. explanatum* and *P. calicophorum*.

As the sucker is said to be somewhat smaller in relation to the length



of the worm in *P. calicophorum* than it is in *P. explanatum*, Table VI has been drawn up by utilising the figures given by Fiscoeder for these two worms and by summarising the figures in Table V of the present paper.

TABLE VI.

	<i>P. explanatum</i> , Fiscoeder	<i>P. calicophorum</i>	<i>P. explanatum</i> , Present material summarised from Table V
Length of worm ... ..	8 mm. to 13 mm.	10 mm. to 15 mm.	6 mm. to 10.1 mm.
No. of worms measured ... ..	?	?	10
Diameter of posterior sucker ... ..	3.5 mm. $\times$ 3 mm.	3 mm. to 4.3 mm.	2.6 mm. to 3.8 mm.
No. of suckers measured ... ..	1	?	10
Ratio of diameter of sucker to length of worm expressed in fractions ...	$\frac{1}{2.3}$	'Not quite $\frac{1}{3}$ '	From $\frac{1}{2.9}$ to $\frac{1}{3.6}$

It seems clear from Table VI that there is no constant relation between the diameter of the posterior sucker and the length of the worm.

Fiscoeder recognises the variation to which the genital apparatus is liable and to which attention has been drawn in *P. explanatum* in the present paper. He states :

'Die Hoden liegen fast neben einander (fig. 29, 30), der vordere etwa in der Mitte des Körpers, mehr dorsal, der hintere mehr ventral (fig. 29, 30 u. 31).'

His references to the figures prove that he is describing the appearance in young worms, which is borne out by his later statement :

'Bei reifen Individuen nehmen sie die ganze, stark verdickte, hintere Hälfte des Thieres bis dicht zum Saugnapf ein (fig. 31).'

He makes no reference to the appearance of the testes in immature *P. explanatum*.

With regard to the excretory bladder, excretory pore, and Laurer's canal, he says :

'In Bezug auf das Lageverhältniss des Excretionporus und der Ausmündungsstelle des Laurer'schen Canals sind häufig gewisse Differenzen zwischen den reifen

und unreifen Individuen zu verzeichnen, die jedoch auch mit der Entwicklung der Hoden und der damit verbundenen stärkern Ausdehnung der hintern Körperpartie in Verbindung gebracht werden können. Während nämlich bei den unreifen Thieren in der Regel die Excretionsblase etwa im Niveau des vordern Randes des Saugnapfes und der Laurer'sche Canal nur 0.3-0.4 mm. dahinter ausmündet (fig. 30, 32 u. Textfig. E), befindet sich der Excretionsporus bei den geschlechtsreifen Thieren meist viel weiter nach vorn, fast in der Höhe des vordern Randes des hintern Hodens (fig. 31), und die Ausmündung des Laurer'schen Canals 0.6-0.8 mm. hinter dem Excretionsporus.'

It is therefore clear that in the case of *P. calicophorum*, Fiscoeder recognised the variation to which these structures are liable, but, as he does not seem to have made a detailed examination of immature material of other species, he failed to realise the general application of this fact. While in agreement with Fiscoeder in the first part of his statement, viz., that the excretory pore and excretory canal vary in their relations to the bladder as age advances, the writer cannot support the latter part of his statement that Laurer's canal opens further behind the excretory pore in gravid worms than it does in immature examples. While agreeing that a variation in this particular can be found, it has been observed to be quite independent of age.

Stiles and Goldberger (1910), apparently basing their definition of *P. calicophorum* on Fiscoeder's paper (1903), write :

'Excretory pore prevesicular.

Testicular fields separate, not median, zones overlap, testes lobate, much smaller than acetabulum; . . . excretory vesicle not narrow but swollen . . .'

From this it is clear they have ignored Fiscoeder's statements quoted above and have taken their definition mainly from characters found in immature worms, which makes it useless for diagnostic purposes. Careful comparison of Fiscoeder's descriptions of the species *P. explanatum* and *P. calicophorum* thus indicates that there are no differences between them, except such as may be explained by differences in age and normal variation; therefore *P. calicophorum* is a synonym of the species *P. explanatum*.

#### *Paramphistomum crassum*, Stiles and Goldberger, 1910.

Stiles and Goldberger (1910) made the species *P. crassum* from three specimens which they state were found with other forms in a bottle from India, the host being *Bos indicus*. They also say that no eggs were seen, so it is probable their material was immature.

On comparing Stiles and Goldberger's description of *P. crassum* with

Fischoeder's description of *P. calicophorum*, the only essential difference between the two is that the excretory pore is dorsal to the excretory bladder in *P. crassum*, and although Stiles and Goldberger assert that the excretory pore is anterior to the excretory bladder in *P. calicophorum*, it is not in agreement with Fischoeder's statement, quoted above, except in fully grown worms, and with this the writer's observations are in agreement. It has also been shown that in *P. explanatum* without eggs the excretory pore is in the position ascribed to it in *P. crassum* by Stiles and Goldberger. When it is recalled that the latter observers saw no eggs it seems probable that they were dealing with young specimens of the same species as Fischoeder.

*Paramphistomum cauliorchis*, Stiles and Goldberger, 1910.

In describing this species Stiles and Goldberger (1910) state that they found three specimens in one bottle and four in another. The host was *Bos indicus* and eggs were not observed. In their description of *P. crassum* Stiles and Goldberger say that it closely resembles *P. cauliorchis*; this is fully borne out by comparing the two descriptions. There is this difference, however, viz., that in *P. cauliorchis* they state that Laurer's canal 'opens slightly to right or left of the median line, 60 to 320 $\mu$  cephalad of the excretory pore,' whereas in *P. crassum* Laurer's canal is described as opening 'about 0.72 mm. caudad of the excretory pore.' But there is some doubt as to the correctness of their observations in *P. cauliorchis* for two reasons. First, in their fig. 62, which is a side view of *P. cauliorchis*, they show Laurer's canal running parallel with the excretory canal, and although the termination of Laurer's canal is not actually shown, it looks as if it would open about the same level as the excretory pore. In the second place, they have made a subgenus *Cauliorchis* of which *P. cauliorchis* is the type, and in their definition of this Stiles and Goldberger state that Laurer's canal is 'caudad to caudo-laterad of the excretory pore, the two pores may be close together . . .' Another point is that in *P. crassum* the testicular fields are said to overlap and in *P. cauliorchis* they are said to be separate, in the latter case being similar to their definition of *P. calicophorum*. The writer has already shown, and is confirmed by Fischoeder, that in *P. explanatum* the testes are separate in young worms, and that in older ones the fields overlap, and although age is the most important factor in influencing this difference, the writer has found young worms of the same

type with the testes overlapping. It is accordingly concluded that there is no difference between *P. crassum* and *P. cauliorchis*.

*Paramphistomum fraternum*, Stiles and Goldberger, 1910.

Railliet, Henry and Bauche (1914a) state that *P. fraternum* is a synonym of *P. explanatum*, and they advance such good reasons for their opinion that it is proposed to accept their conclusion without further discussion. In the light of the present investigation, however, there is one point of interest in the conclusion of Railliet, Henry and Bauche, viz. : Stiles and Goldberger give as one of the distinguishing characters of *P. fraternum* that the genital pore is opposite the anterior sucker in this species, whereas it is posterior to the gut fork in *P. explanatum*. From this it may be inferred that the above observers do not consider the position of the genital pore of any specific value. Moreover, Fischöder also seems to be of the same opinion, as Railliet, Henry and Bauche state that they obtained his opinion on their material and that he agreed with them as to its identity.

*Paramphistomum siamense*, Stiles and Goldberger, 1910.

This species is differentiated from *P. cervi* and *P. fraternum* by Stiles and Goldberger and is therefore considered new by them. In their diagnosis from *P. cervi* they use the difference in size of the posterior sucker, the position of the genital pore, and the shape of the worm, but they omit the one real distinguishing character, viz., the different relations of the testes to one another in the two species. *P. fraternum* is distinguished from *P. siamense* because the worms are said to be of different shape; the posterior sucker of *P. fraternum* is relatively smaller than it is in *P. siamense*; and the genital pore is proportionately nearer to the anterior end in *P. fraternum*. The question of small differences in shape may be disposed of by reference to Plate VI, fig. A, which is a photograph of several specimens of *P. explanatum*, all of which were taken from one bottle. With regard to the position of the genital pore, this is given as midway between that described for *P. explanatum* and *P. fraternum*, so the opinion of Railliet, Henry and Bauche (1914a), which has been quoted under the discussion of the latter species, is of equal value in this case. The relative difference in size of the posterior sucker in the two species requires more careful consideration.



In the case of *P. fraternum* Stiles and Goldberger say that only two specimens 'in poor condition' were available. The length of one of these specimens is given as 9.75 mm. when in alcohol, and the same worm measured 8.94 mm. after imbedding and sectioning. The diameter of the posterior sucker of this worm is given as 3.75 mm. in the antero-posterior and 3.25 mm. in the transverse direction. In the case of *P. siamense* the length of two specimens measured in glycerine alcohol is given as about 6 mm. and 9 mm. respectively. The posterior sucker was measured in three 'press preparations,' and the diameters of the three are given as 3.5 mm., 4 mm. and 5 mm. respectively. It is not quite clear what is meant by press preparations, but it is assumed that it means that the worms were subject to pressure before being measured. The writer has found that pressure between slides is of great advantage in obtaining a clear view of the internal organs in carbolic cleared specimens, but it was found that measurements of worms while under pressure in this way were quite unreliable. This was especially the case with regard to the posterior sucker; because, the worms being much thicker at the posterior end than at the anterior end, when pressure was applied a considerable flattening and consequent distortion was caused to the hinder end before the anterior end was subject to any pressure. It is therefore clear that the posterior portions of the worms were always relatively more distorted than the anterior portions, and hence the structures in the hinder part were relatively greatly increased. It seems that this is what has occurred in Stiles and Goldberger's case, and that owing to their failure to recognise this fact they have been led to ascribe specific value to what is in reality an artificial condition. Indeed, from the writer's experience, it is surprising that the difference in size of the posterior sucker in *P. fraternum* and *P. siamense* was not greater than they actually found it. It is therefore considered that the small difference between the relative size of the posterior suckers in these two worms should not be regarded as of any diagnostic value, and in consequence the two worms are probably identical with one another and with *P. explanatum*.

As a result of the above investigation it is concluded that *P. calicophorum*, *P. crassum*, *P. cauliorchis*, *P. fraternum* and *P. siamense* are all synonyms of *P. explanatum*.

In all the species of the genus *Paramphistomum* that have been dealt with up to the present the excretory pore is anterior to the opening of



Laurer's canal. Those species in which Laurer's canal opens anterior to the excretory pore will now be considered. As the writer has not had an opportunity of examining the following species, the description is in each case summarised from the original.\*

*Paramphistomum orthocoelium*, Fiscoeder, 1901.

SYNONYMY:—

*Paramphistomum dicranocoelium*, Fiscoeder, 1901.

*Paramphistomum streptocoelium*, Fiscoeder, 1901.

*Paramphistomum scoliocoelium*, Fiscoeder, 1904.

*Paramphistomum parvipapillatum*, Stiles and Goldberger, 1910.

*Paramphistomum shipleyi*, Stiles and Goldberger, 1910.

First found in the stomach of a *Bos kerabau* that died in Berlin.

The following notes were made from Fiscoeder (1903).

Cuticular papillae are present on the anterior end of the worm. The oesophagus is twice as long as the anterior sucker, and is surrounded by especially large and numerous cells. The muscle walls of the oesophagus are not thicker than in most other species of the genus. The gut caeca are not wavy, they are almost straight; they lie about midway between the dorsal and ventral surfaces on each side of the worm, and they end 0.5 mm. to 1 mm. in front of the posterior sucker; the diameter of the caeca is 1 mm. to 1.2 mm. dorso-ventrally and  $500\mu$  to  $600\mu$  transversely. With the exception of the vitellaria, the genital organs exhibit no special characters. The genital papilla is well developed and almost fills the atrium, being in most cases slightly protruded. The testes lie one behind the other in the posterior half of the worm; their size and the distinctness of their lobulation is variable. The vitellaria, however, consist of round to oval groups of follicles nearly uniform in size and about  $300\mu$  in diameter. As a rule, they are in a single row along the ventral border of the caeca on each side except near the posterior end, where they are grouped to the number of from four to six follicle groups which lie close together. The eggs are  $105\mu$  to  $115\mu$  in length by  $60\mu$  to  $65\mu$  in breadth.

\* Since completion of this paper the writer has been able to examine a collection of a few specimens of *P. orthocoelium* from the stomach of a sheep at Hong Kong. Although it is necessary to section the worm to see the course of Laurer's canal, simple clearing in carbolic acid is sufficient to establish the identity of this species. It is easily distinguished from *P. cervi* by the vitellaria which in this species are arranged in very numerous comparatively small groups of follicles, never more than about  $300\mu$  in diameter, and showing a marked tendency to encroach on the dorsal and ventral surfaces of the worm, whereas in *P. orthocoelium* the number of follicle groups is much less, they are considerably larger, being up to  $700\mu$  in diameter, and are limited almost exclusively to the lateral fields external to the gut caeca.

*Paramphistomum dicranocoelium*, Fiscoeder, 1901.

The following notes are taken from Fiscoeder (1903).

Cuticular papillae were not seen on the anterior end. The worm is very near *P. orthocoelium*. The oesophagus is shorter than in *P. orthocoelium*, its extreme length being one and a half times as long as the anterior sucker, and instead of becoming thicker only quite close to the gut fork the muscle wall commences to thicken about its middle, and from this point it gradually increases from  $20\mu$  to  $60\mu$ - $75\mu$  at the posterior end. The gut caeca are straight, but differ from *P. orthocoelium* in being nearer to the dorsal than to the ventral surface, and they are only  $250\mu$  to  $350\mu$  in diameter; they end about 1 mm. in front of the posterior sucker. The genital papilla is well developed, but is strongly retracted as a rule. The vesicula seminalis is larger and lies farther towards the dorsal surface, but on the other hand the pars muscosa is not so long as it is in *P. orthocoelium*, and the pars prostatica is somewhat shorter also. The ductus hermaphroditicus may be a fairly broad pear-shaped cavity, or, when the papilla is strongly retracted, the ductus hermaphroditicus may be merged in the genital atrium. The vitelline glands are arranged in similar groups to *P. orthocoelium*, but they lie as a rule in two rows and show no special grouping behind the ends of the caeca. The anterior ends of the vitellaria may be at different levels. The other genital organs are approximately the same as in *P. orthocoelium*. The eggs measure  $145\mu$  to  $150\mu$  in length and  $75\mu$  to  $80\mu$  in breadth.

*Paramphistomum streptocoelium*, Fiscoeder, 1901.

The following notes were taken from Fiscoeder (1903).

Cuticular papillae are present on the anterior end as in the case of *P. orthocoelium*. The oesophagus is only about as long as the anterior sucker; the gut caeca are strongly convoluted and end opposite the base of the sucker. The genital atrium is distinguished by the presence of a ring-like prominence on its inner wall which divides the atrium into a small ventral and a large dorsal chamber. The testes, as well as varying in proportion to the size of the worm, may be of different sizes in the same worm and apparent partial atrophy of these organs has been noted in gravid worms. The position of the testes is the same as in *P. dicranocoelium*. The female genitalia only show slight differences from the previous species.

The vitellaria are confined to the sides of the worm and stretch from the gut fork in front to the posterior sucker behind as in *P. dicranocoelium*. The follicles are in groups similar to the previous species ( $300\mu$  to  $700\mu$  in diameter), but they are present in larger numbers and are therefore smaller in size than in the two previously named species. The anterior limits of the vitellaria may be at different levels on the two sides as in *P. dicranocoelium*. The eggs measure  $105\mu$  to  $115\mu$  in length by  $60\mu$  to  $65\mu$  in breadth.

*Paramphistomum scoliocoelium*, Fischoeder, 1904.

The following notes are taken from Fischoeder (1904).

The musculature of the oesophagus is similar to that of *P. dicranocoelium*. The gut caeca are less wavy than in *P. streptocoelium*, and they are nearer to the dorsal than the ventral surface as in *P. dicranocoelium*. The genital pore is, as a rule, widely open. The vitellaria are similar to the other three species; they are composed of coarse follicles which lie at the sides of and external to the gut fork, from about the level of the gut fork in front opposite to the base of the posterior sucker behind. The eggs are  $135\mu$  to  $145\mu$  in length by  $65\mu$  to  $75\mu$  in breadth.

*Paramphistomum parvipapillatum*, Stiles and Goldberger, 1910.

The following notes are taken from Stiles and Goldberger (1910).

The cavity of the anterior sucker is furnished with moderate sized papillae. The oesophagus is estimated to be not shorter than the anterior sucker and it exhibits a thickening of its muscle wall in its posterior half. The gut caeca are slightly wavy and end opposite the middle of the posterior sucker. The genital papilla is embraced by a ring-like collar beset with fine papillae. External to this collar is another ring marked off from it by a groove, and this groove may not be present in cases with the genital papilla protruded. The vitellaria consist of numerous follicles lying at the sides of, above, and below the gut caeca; the gland groups on the left-hand side extend slightly further inwards than those on the right hand. The vitellaria extend from the hinder end of the anterior sucker in front to opposite about the middle of the posterior sucker behind. The eggs measure  $135\mu$  in length by  $67\mu$  in diameter.

*Paramphistomum shipleyi*, Stiles and Goldberger, 1910.

The following notes are from Stiles and Goldberger (1910).

The entrance to the anterior sucker and the sucker itself are lined with small papillae. The muscle wall of the oesophagus begins to grow thicker from about the middle of its length towards the posterior end. The caeca are wavy and end about the level of the anterior border of the cavity of the posterior sucker. The diameter of the gut varies at different points along its course, there being marked dilatations followed by constrictions. The testes are one behind the other, but overlap slightly. The ductus ejaculatorius opens close to, but separate from and just above the opening of the uterus. The space into which this ductus ejaculatorius opens is narrow and slit-like.

‘From this space a short duct passes ventrad and may be regarded as piercing the axial region of a mushroom-like structure (figs. 123-126) to open into another slit-like atrium somewhat larger, however, than the one into which the male and female ducts open. A duct about  $30\mu$  in diameter leads from this atrium and apparently pierces a stout conical papilla, which may be regarded as the genital papilla, to open into a small genital atrium which connects with the exterior by the genital pore.’

The eggs are about  $135\mu$  in length by  $71\mu$  in breadth.

In conducting a critical examination into the specific value of the above worms, it is in the first place obvious that the points used for differentiation are all similar to those used in the group of worms that have been considered under *P. cervi*. But in the present instance the differences are even smaller than in the former case. For instance, differences in length of the oesophagus greater than in these six species have been noted in all species examined. This is only to be expected when it is realised that the oesophagus is well supplied with muscle, contraction or relaxation of which can easily account for the differences observed. The amount of increase of thickness towards the posterior end of this organ has also been shown to vary considerably in the same species. The point of termination of the gut caeca only varies very slightly in the above six species; this character has been found to have no diagnostic value in other cases, and that in the present instance it is equally valueless is appreciated, when it is noticed that in *P. scolicoelium*, Fischöder shows the caeca ending in front of the posterior sucker in fig. 7, and behind the anterior border of the sucker in fig. 8, although in the text he says they end in front of the posterior sucker. It has also been found that slight differences in the



amount of convolution of the caeca, as well as in their diameter, are of no value. A moment's reflection will explain the reason for this. The caeca are hollow structures furnished with muscular walls and it will thus be obvious that the amount of convolution may vary considerably according to the degree of contraction of the muscular wall of the gut and of the whole worm. The value of slight differences in the distribution of the vitellaria has also been shown of no use for specific diagnosis in all other species, and that the same probably applies in the present series of worms is borne out by comparison of the statements in regard to these glands in several of the worms. For example, Fischöder states that *as a rule* the gland groups are in a single row in *P. orthocoelium*, and in *P. dicranocoelium* they are *as a rule* arranged in a double row; but as he does not state what the exceptions to these rules are, all that one can infer is that the vitellaria are subject to some variation in both these species and cannot, therefore, be accorded any specific value. The liability to variation of the vitellaria is also borne out by the following contradictory statements by the same author: in *P. orthocoelium* he says the vitelline follicles are in groups, each of which is about  $300\mu$  in diameter; in *P. dicranocoelium* they are similar to the above; and in *P. streptocoelium* they are in groups similar to the above, but having a diameter of from  $300\mu$  to  $700\mu$ ; whilst in the next sentence he states they are present in *P. streptocoelium* in far greater numbers than in the two previous species and are in consequence of smaller size. With regard to the genital apparatus, there is no evidence of any allowance having been made for variations due to protrusion or retraction of the genital papilla at the time of fixation. For example, the genital papilla of *P. orthocoelium* is described as being well developed and in most cases filling the atrium; in *P. dicranocoelium* the papilla is said to be well developed and in most cases strongly retracted; in *P. streptocoelium* a ring-like prominence divides the atrium into two chambers; in *P. scoliocoelium* the genital pore is stated to be, as a rule, widely open; in *P. parvipapillatum*, the papilla is described as being embraced by a collar-like ring beset with minute papillae; and in *P. shipleyi* from the single sectioned example that they studied Stiles and Goldberger describe a complicated arrangement of ducts and atria which, in the writer's opinion, is only a description of a worm with the papilla in strong retraction such as can be found in any species, if enough specimens are examined. All the various descriptions of genital papilla and atrium described above have been seen by the writer in all the species in which he has had sufficient



material to examine a long series of mature worms. It is therefore considered that in the present case they cannot be regarded as specific differences. The only other point relates to the small papillae described as present in some species of this group of worms and absent in others. In all other species they have been found to vary to a great extent, and accordingly it is probable that in the present case they vary in the same way, and are in consequence of no diagnostic value.

The evidence obtained from the examination of closely allied species, indicates that the points on which the differentiation of the above six species is based, are of no specific value. It is therefore considered that *P. dicranocoelium*, *P. streptocoelium*, *P. scoliocoelium*, *P. parvipapillatum* and *P. shipleyi* are synonyms of *P. orthocoelium*.

*Paramphistomum buxifrons*, Leiper, 1910.

Host :—*Hippopotamus* sp. Location :—Stomach. Locality :—Uganda.

According to Leiper, *P. buxifrons* can readily be distinguished by its leaf-like shape which resembles the leaf of a box tree. A large example may measure 5 mm. in length by 3 mm. in breadth and be only 0.4 mm. in thickness at the middle of the body. The testes also are a distinguishing character, as they are not lobed and they are placed diagonally in the posterior part of the worm. The only other species with testes not lobed are *P. liorchis* and *P. wagandi* and in these cases the testes are one behind the other, but in *P. buxifrons*, Laurer's canal opens in front of the excretory pore, whilst in *P. liorchis* Laurer's canal is behind the excretory pore.

*Paramphistomum wagandi*, Leiper, 1910.

Host :—*Hippopotamus* sp. Location :—Stomach. Locality :—Uganda.

The testes are not lobed and are placed one behind the other, and the worm is therefore very similar to *P. liorchis*; the two species are distinguished by the position of the opening of Laurer's canal, which is in front of the excretory pore in *P. wagandi*, whereas it is behind the excretory pore in *P. liorchis*.

In his descriptions of these species, Leiper lays down by definite measurement the exact size and position of the various organs, the appearance of the genital atrium, the shape of the excretory bladder, etc. In most cases these particulars appear to have been obtained from a single sectioned

specimen. This fact is considered unfortunate, because slight variations from the data given by Leiper are almost certain to be found when these worms are more fully known, and will in all probability lead to a multiplication of species on the ground of slight variations, in the same way as seems to have occurred in most of the other species.

Genus *Cotylophoron*, Stiles and Goldberger, 1910.

*Definition.*—*Paramphistominae*: with a genital sucker distinctly marked off from the subcuticular muscle layer.

Type species: *Cotylophoron cotylophorum* (Fischöeder, 1901), Stiles and Goldberger, 1910.

#### KEY TO SPECIES

Laurer's canal opens posterior to excretory pore ... ..	<i>C. cotylophorum</i>
Laurer's canal opens anterior to excretory pore ... ..	<i>C. minutum</i>

*Cotylophoron cotylophorum* (Fischöeder, 1901), Stiles and Goldberger, 1910.

#### SYNONYMY:—

*Paramphistomum cotylophorum*, Fischöeder, 1901.

*Cotylophoron indicum*, Stiles and Goldberger, 1910.

First found in the stomach and intestines of *Bos* sp. in German East Africa.

The material available for study in the present case consisted of the following collections:—

1. Ten bottles from the stomach of bullocks killed at Sierra Leone, West Africa.
2. Four bottles from the stomach of buffaloes (*Bubalus* sp.) in the Upper Shire River, Nyasaland.
3. Four bottles from the stomach of two nswala (*Aepyceros melampus*) shot in the Upper Shire River District, Nyasaland.
4. One bottle from the stomach of a Pagan dwarf bull from Ilorin, Northern Nigeria.
5. One bottle from the stomach of a waterbuck (*Cobus* sp.) from Zeref, Khartoum.
6. One bottle from the stomach of a hartebeest (*Bubalis* sp.) from Nyasaland.
7. One bottle from the stomach of an antelope sp. (?) from Rhodesia.
8. One bottle from the stomach of an antelope sp. (?) from Nyasaland.

In some of these bottles were many hundreds of specimens, so ample material was available for examination.

The species *C. indicum* was made by Stiles and Goldberger from six specimens. They state that no eggs were observed, so it is probable that their material was immature.

The differences between *C. cotylophorum* and *C. indicum* are summarised by Stiles and Goldberger as follows :—

'*Cotylophoron indicum* comes close to *C. cotylophorum* from which it differs chiefly in the structure of the oesophagus, which is provided with a bulbous thickening in the latter species, but is without it in the former. The two differ also in the details of structure of the copulatory apparatus and in the position of the genital pore. In *C. indicum* the genital sucker is less sharply delimited, projects less, has a much smaller genital atrium, and the genital pore is decidedly postbifurcal; on the other hand, in *C. cotylophorum* the genital sucker is sharply marked, with rim prominently bulging the venter, with a relatively roomy genital atrium and with the genital pore in the bifurcal zone.'

It should be noted that Fiscoeder (1903) in his description of *P. cotylophorum* says the uterus is strongly convoluted and it is full of eggs, showing that his specimens were mature.

The following points have been worked out from the examination of many specimens of *C. cotylophorum*, either cut and mounted in serial sections, or examined whole in carbolic acid.

*Oesophagus.* In their description of the species *C. indicum*, Stiles and Goldberger state that the walls of the oesophagus are thick, but give no other important details of its characters. In the present instance, the thickness of the muscle walls of the oesophagus, as well as its length and direction, were found to be very variable. Although it was much more distinct in some cases than in others, there was always a gradual increase in thickness of the muscle wall of the oesophagus from the anterior end towards the posterior end, in exactly the same way as described in *P. cervi*. Fig. 7 represents camera lucida drawings of eleven specimens of *C. cotylophorum* cut in sagittal section. It will be noted that in figs. K and A, the oesophagus is approximately the same as in fig. 45 by Stiles and Goldberger, which is a drawing of *C. indicum*. Now, taking the remaining drawings in fig. 7 in the following order D, B, E, C, L, F, G, H, it will be observed that as the oesophagus gradually increases in length, its posterior extremity becomes more and more bulbous. Drawings C, L, G, and F are very similar to Fiscoeder's fig. 38, which is a drawing of *C. cotylophorum* cut in the sagittal plane. In addition to the above characters it was noted that the worms from which drawings J and K were made contained no eggs, whilst all the others had eggs in the uterus, those with the longest oesophagus having the most eggs. It seems probable, therefore, that the differences in the oesophagus in *C. indicum* and *C. cotylophorum* are really due to differences of age. This is all the more likely when it is remembered that Stiles and Goldberger's material did not contain eggs,

and that Fiscoeder's did. Two other points are also well illustrated in the above series of drawings, viz., that the course and length of the oesophagus are subject to considerable variation, and that the position of the genital pore varies in relation to the gut fork to such an extent that neither of these points is reliable for distinguishing between *C. indicum* and *C. cotylophorum*.

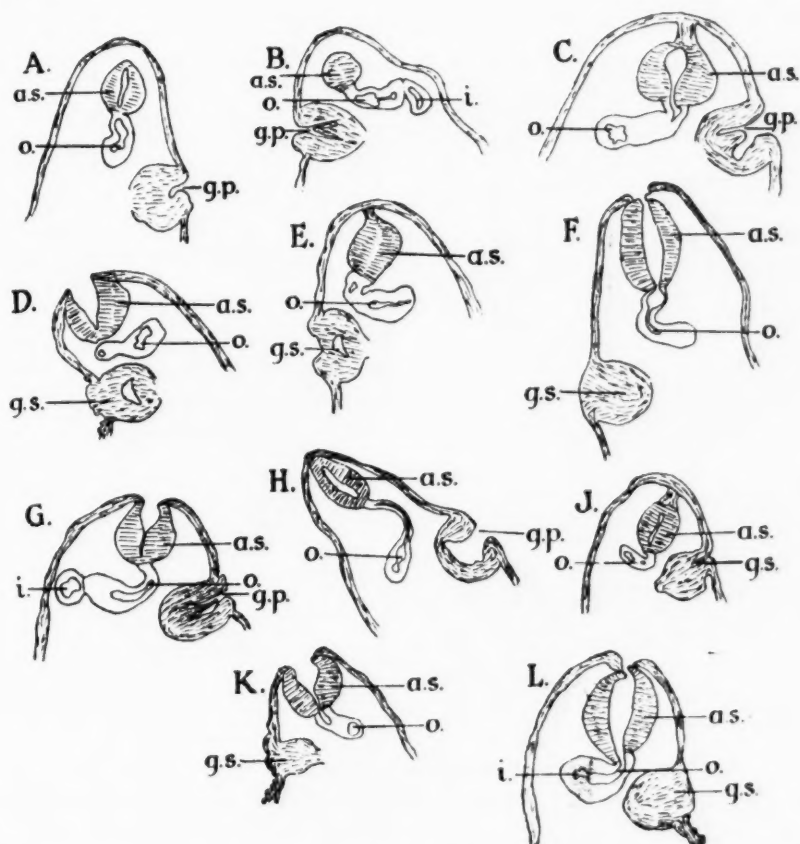


FIG. 7. *Cotylophoron cotylophorum*. Sagittal section through the anterior ends of eleven specimens to show oesophagus and genital sucker. a.s.—anterior sucker; g.p.—genital pore; g.s.—genital sucker; i.—intestine; o.—oesophagus.  $\times 12$ .

**Genital apparatus.** The differences in size, and degree of extrusion or retraction of the genital sucker and papilla, with consequent changes in the genital atrium, were almost as numerous as the specimens examined. This is well illustrated in figs. 8 and 9, which show such great differences in appearance that at first sight it might be considered that different species are being dealt with, but it should be borne in mind that the above examples have been chosen for this very reason, and that many specimens have been examined showing all possible intermediate stages, thus rendering it practically certain that it is only a question of individual variation. Fig. 8, A1 and A2, are two sections of the same worm, and they have been included because Fiscoeder (1903) states that the male and female



openings have always been noted by him to be separate in *C. cotylophorum* as shown in fig. 8, A2, which closely corresponds to Fiscoeder's fig. 38. But a later section (fig. 8, A1) showed the male and female ducts uniting within the substance of the genital papilla and opening on the surface by

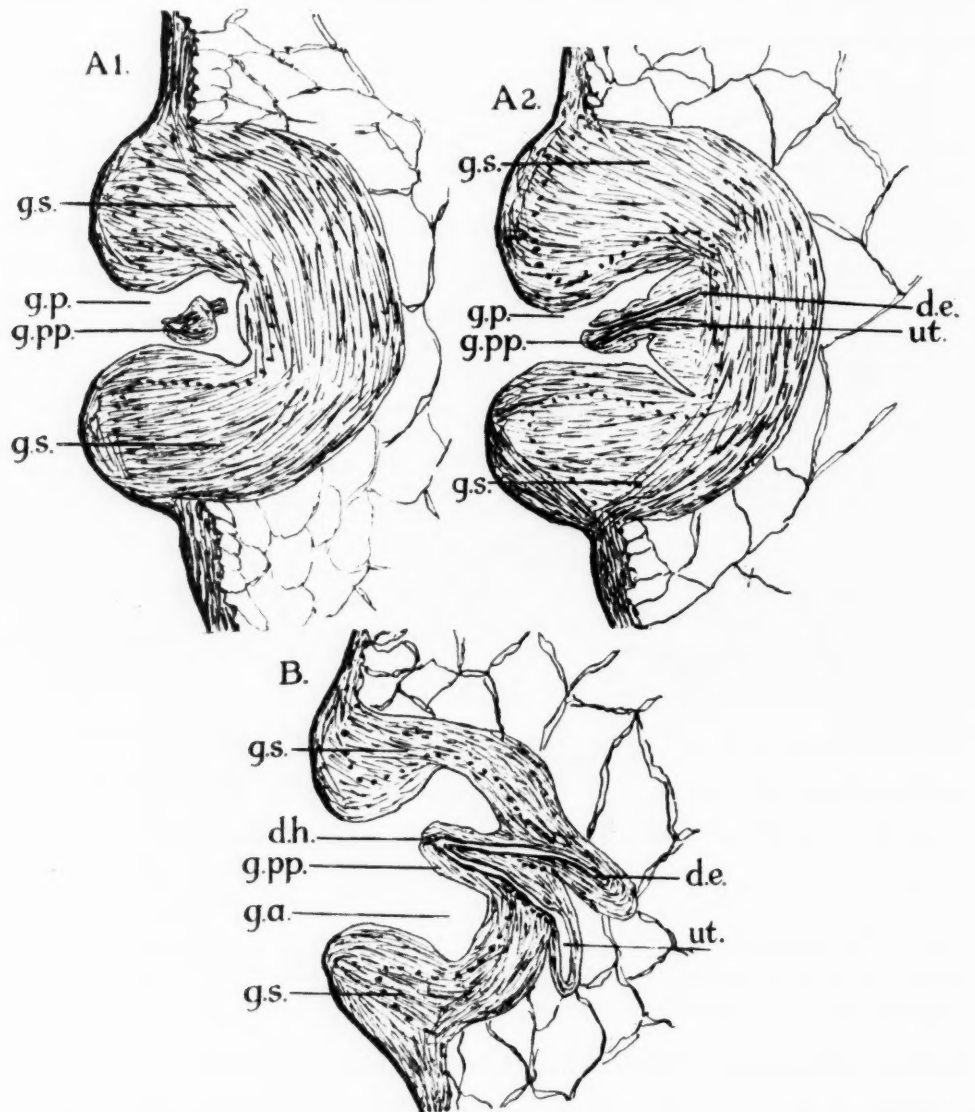


FIG. 8. *Cotylophoron cotylophorum*. Sagittal section through genital sucker. A1—Section showing tip of genital papilla with male and female ducts uniting in the usual way. A2—Section of same specimen with male and female ducts apparently opening separately, because tip of genital papilla is not seen. B—Section of another specimen with genital papilla lying in a wide atrium. d.e.—ductus ejaculatorius; d.h.—ductus hermaphroditicus; g.a.—genital atrium; g.p.—genital pore; g.pp.—genital papilla; g.s.—genital sucker; ut.—uterus.  $\times 45$ .

a common duct. It is therefore clear that there is no essential difference between this worm and other species with regard to the termination of the male and female ducts. Fig. 8, B, shows how different the genital pore may appear if it is relaxed with a large genital atrium and a patulous genital pore, but in sections not passing through the genital atrium the genital



sucker was seen to be just as thick as in other cases. In fig. 9 two very different appearances of the genital apparatus are shown. Fig. 9, A, shows the whole genital sucker protruded beyond the surface of the worm, while in 9, B, it is deeply retracted within the body. It will be noted that in fig. 9, A, the subcuticular muscle extends past the base of the genital sucker in an apparently unbroken column of fibres, and that even opposite its base the parenchyma is not sharply marked off from the sucker, whereas in fig. 9, B, with the sucker deeply retracted, this separation is quite distinct. The conclusions drawn from these two figures are that the demarcation of

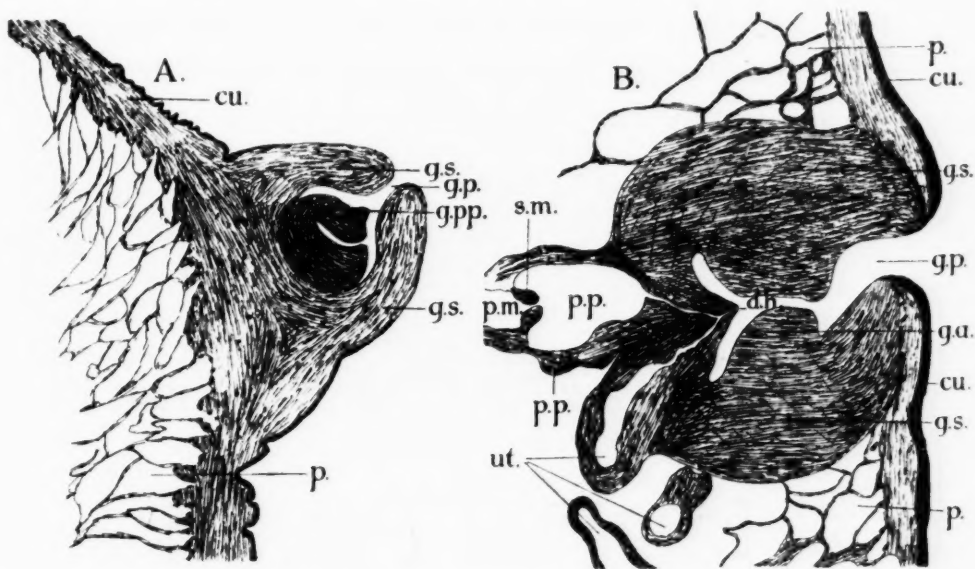


FIG. 9. *Cotylophoron cotylophorum*. Sagittal section of genital sucker of two specimens. A—Genital sucker fully extruded.  $\times 40$ . B—Genital sucker fully retracted  $\times 60$ . cu.—cuticle; d.h.—ductus hermaphroditicus; g.a.—genital atrium; g.p.—genital pore; g.p.p.—genital papilla; g.s.—genital sucker; p.—parenchyma; p.m.—pars muscosa; p.p.—pars prostatica; s.m.—sphincter muscle; ut.—uterus.

the sucker from the parenchyma in this genus is more apparent than real, and that it is probably brought about by the subcuticular muscle and the contiguous parenchyma being stretched around the sucker when it is in the retracted condition. Comparison of the shape and size of the atrium in the drawings will make it sufficiently clear without further comment that minute descriptions of the shape and number of chambers in this atrium cannot be regarded as of any value for specific diagnosis. The conclusion arrived at from consideration of the above facts is that Stiles and Goldberger, in describing *C. indicum* from six specimens with no eggs in the uterus, were in all probability dealing with immature specimens of *C. cotylophorum*.

In addition to the above points, which were worked out in sectioned

specimens, evidence of variation in other characters was obtained by the examination of eighty specimens cleared in carbolic acid and examined whole.

*Gut caeca.* The amount of convolution of the caeca varies considerably, as does their point of termination. The most usual position for the caeca to end in this species is about opposite the middle of the posterior sucker, but in some cases they both ended well in front of the anterior border of the posterior sucker, and in others they nearly reached the posterior border of the posterior sucker. Between these two extremes all intermediate stages were found, and in a few instances the caeca on the two sides of the same worm ended at different levels. As a rule, the final turn of the caeca was directed dorsally, but this was by no means invariable, as is clearly shown in fig. 10.

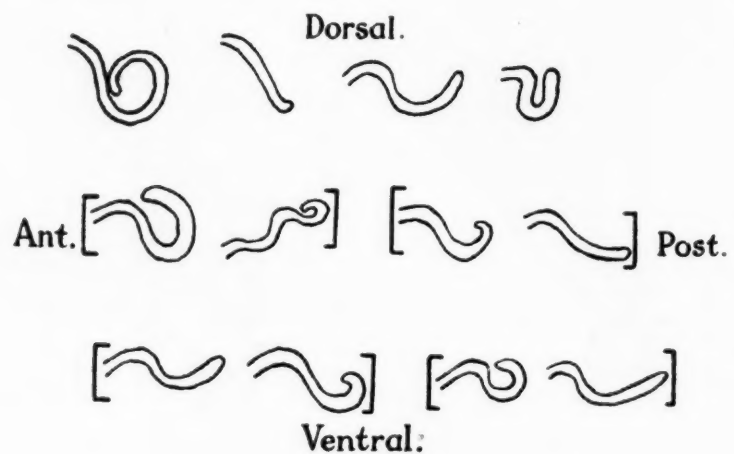


FIG. 10. *Cotylophoron cotylophorum*. Drawings showing various forms of termination of gut caeca. These figures are not to scale, and are drawn from carbolic cleared specimens. Those in brackets are the two caeca from a single worm.

*Vitellaria.* The distribution of the vitelline glands and the number of collections of follicles in each gland showed a remarkable degree of variation. The glands commenced anteriorly anywhere from about opposite the middle of the anterior sucker to a point slightly behind the genital pore; as a rule, they extended posteriorly to a little behind the termination of the gut caeca, but in a few instances groups of follicles were seen extending to the extreme posterior end of the worm and surrounding the opening of the posterior sucker in the same way as in all the other species examined. The degree of extension inwards on the dorsal and ventral surfaces varied in the same way as in *P. cervi*. The anterior extension of the gland was often different on the two sides, and in one extreme instance the vitelline gland on the left side began in front of the genital pore and ended just

behind the corresponding caecum, but on the other side the gland was composed of a closely packed collection of follicle groups, which commenced opposite about the middle of the hinder testis and ended opposite the middle of the posterior sucker, being completely confined to the outer side of the caecum of that side.

*Excretory system.* The change in the relations of the excretory pore to the excretory bladder in worms of different age, which has been referred to in *P. cervi* and *P. explanatum*, has been found to hold good in the case of *C. cotylophorum*. This is clearly shown in fig. 11, which represents the

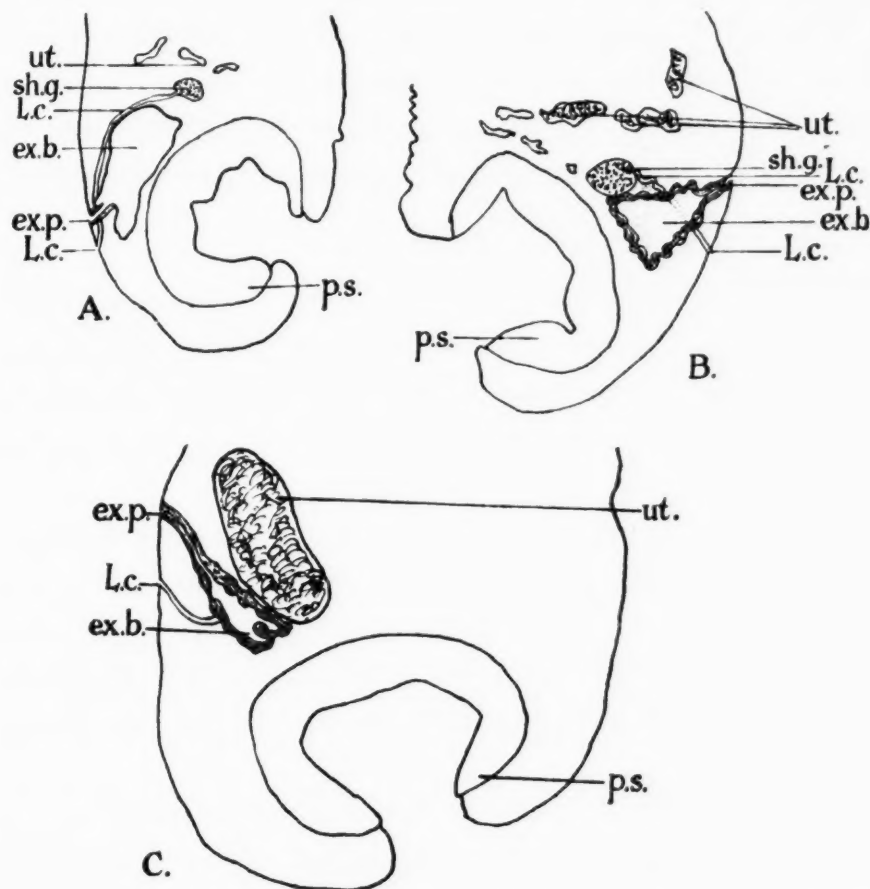


FIG. 11. *Cotylophoron cotylophorum*. Sagittal sections of three specimens near the mid-line to show the alteration in course of the excretory canal with increasing age. A—Immature worm. B—Partly gravid worm. C—More fully gravid worm. *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *L.c.*—Laurer's canal; *p.s.*—posterior sucker; *sh.g.*—shell gland; *ut.*—uterus  $\times 18$ .

posterior ends of three specimens of *C. cotylophorum* of different ages. These figures also emphasise the fact that the shape of the excretory bladder is very variable.

Leiper (1910) described two worms from the hippopotamus, which he placed in the genus *Paramphistomum*, but both of them have a definitely

delimited muscular thickening round the genital pore. Following the classification of Stiles and Goldberger (1910) these worms should be placed in the genus *Cotylophoron*. The following are the principal distinguishing features of these species according to Leiper's descriptions.

*Cotylophoron minutum* (Leiper, 1910).

SYNONYMY:—*Cotylophoron sellsi* (Leiper, 1910).

First found in the stomach of a *Hippopotamus* sp. in Uganda.

The testes are small and oval in shape and they lie one behind the other, being separated by a space about equal to the diameter of a single testis. Although it is not stated in the text, it is assumed from Leiper's fig. 32 that the testes are not lobed. Laurer's canal opens in front of the excretory pore. This worm differs from *C. cotylophorum* in that the testes are not lobed and that Laurer's canal opens in front of the excretory pore.

*Cotylophoron sellsi* (Leiper, 1910).

This species is very similar to *C. minutum*, the only differences being that it is larger and that its testes are relatively larger and closer together than in *C. minutum*. Leiper also describes a large genital atrium, but as he says that he made his observations from a single sectioned specimen this cannot be regarded as of specific importance.

The only differences of importance therefore between the two species *C. sellsi* and *C. minutum* are those of size, and as quite as big a range of variation has been found in several series of *C. cotylophorum* examined by the writer, both with regard to the size of the whole worms and of the testes, it is considered probable that *C. minutum* and *C. sellsi* are identical.

Sub-family CLADORCHINAE, Fiscoeder, 1901.

Definition.—*Paramphistomidae*: oral sucker with a pair of diverticula.

KEY TO GENERA

1.	Cirrus pouch absent	...	...	...	...	...	<i>Pseudodiscus</i>
	Cirrus pouch present	...	...	...	...	...	2
2.	Testes smooth	...	...	...	...	...	<i>Balanorchis</i>
	Testes lobed or branched	...	...	...	...	...	3
3.	Testes lobed	...	...	...	...	...	<i>Pfenderius</i>
	Testes branched	...	...	...	...	...	4
4.	Testes each consist of four branches like a cross	...	...	...	...	...	<i>Gbiorchis</i>
	Testes consist of many long irregular branches	...	...	...	...	...	5
5.	Main portion of uterus posterior to testes, terminal portion runs ventral to testes	...	...	...	...	...	<i>Taxorchis</i>
	Main portion of uterus arches dorsally over testes, terminal portion anterior to testes (as in <i>Paramphistomum</i> )	...	...	...	...	...	<i>Cladorchis</i>



Genus *Watsonius*, Stiles and Goldberger, 1910.

Careful comparison of the definitions of *Watsonius* and *Pseudodiscus*, as given by Stiles and Goldberger, reveal little difference between the two.

In the definition of *Pseudodiscus* they state the genital pore is behind the gut fork; a ductus hermaphroditicus is present; the oral sucker is 'prominently constricted at equator'; the oesophagus has no muscular thickening; and the testes lie side by side near the middle of the worm and are 'cauliflower-like,' which means lobed with lobular subdivisions.

The same points in *Watsonius* are described as follows:—The genital pore is in front of the gut fork; a ductus hermaphroditicus is apparently absent; no mention is made as to whether the oral sucker is constricted or not; the oesophagus has a distal thickening of the muscle layer; and the testes are lobulate lying almost one behind the other. All the other points cited are the same in the two genera, or else are not of any value.

It is repeatedly shown in the present paper that the position of the genital pore in front or behind the gut fork is not even of specific value. As the constriction of the oral sucker is only alluded to in one genus, this point may be discarded. The differential diagnosis thus rests on the apparent absence of a ductus hermaphroditicus, the presence of an oesophageal thickening and the tandem arrangement of the testes in *Watsonius*, against the presence of a ductus hermaphroditicus, the absence of an oesophageal muscular thickening, and the lateral position of the testes in *Pseudodiscus*. In the sub-genus, *Hawkesius* of *Pseudodiscus*, however, Stiles and Goldberger state that there is a pronounced thickening of the posterior part of the oesophagus, and the testes are tandem. In respect of these two characters, the definition of *Pseudodiscus* as given by them cannot be correct. Thus, all the differential points between the genera *Pseudodiscus* and *Watsonius* are eliminated, except the doubtful absence of a ductus hermaphroditicus in the latter. The fact that Stiles and Goldberger in erecting the genus *Watsonius* had only a single specimen cut in transverse section, which they borrowed from Shipley, makes this point of very doubtful value, because it is so difficult to make out a ductus hermaphroditicus in sections of this nature, that it is unsafe to rely on them without confirmation from examination of sagittal sections. Leiper (1913), however, in a short record of *W. watsoni* figures the ductus ejaculatorius and uterus opening separately on the end of a prominent papilla, there being a narrow tongue-like process between the two; thus he apparently confirms



Stiles and Goldberger's statement that a ductus hermaphroditicus is absent. In the same paper, Leiper gives a drawing of *Gastrodiscoides hominis* in which the genital papilla and the two ducts are shown to be practically identical with his figure of *W. watsoni*; the writer has examined sagittal sections of three specimens of *G. hominis* and found a papilla with a long ductus hermaphroditicus in the first specimen (fig. 29), a much shorter ductus hermaphroditicus in the second, and in the third there was no papilla at all and the male and female ducts opened separately at the bottom of a deep atrium (fig. 28). It seems probable that the same variation could be found if a number of *W. watsoni* were examined; this is all the more likely when it is remembered that the same range of variations has been found in every species which the writer has been enabled to examine.

This discussion indicates that there are no clear differences between the genera *Pseudodiscus* and *Watsonius*, and that the latter should be merged in the former.

It should be noted that Railliet, Henry and Joyeux (1912), in recording *Watsonius watsoni* from *Cercopithecus callitrichus*, draw attention to the great similarity between this worm and *Hawkesius*, the sub-genus of *Pseudodiscus* created by Stiles and Goldberger (1910).

#### Genus *Pseudodiscus*.\*

SYNONYMY :—*Watsonius*, Stiles and Goldberger, 1910.

*Definition*.—*Cladorchinae*: without a cirrus pouch, and testes lobed.

Type species *Pseudodiscus collinsi* (Cobbold, 1875), Sonsino, 1895.

#### KEY TO SPECIES

Testes side by side	...	...	...	...	...	...	...	<i>P. collinsi</i>
Testes tandem	...	...	...	...	...	...	...	<i>P. hawkesii</i> and <i>P. watsoni</i>

*Pseudodiscus collinsi* (Cobbold, 1875), Sonsino, 1895.

SYNONYMY :—*Pseudodiscus stanleyi* (Cobbold, 1875), Sonsino, 1895.

First found in the colon of the horse in India.

The material investigated all came from India, and was as follows :—

\* Stiles and Goldberger divide the genus *Pseudodiscus* into two sub-genera, viz., *Pseudodiscus*, and *Hawkesius*, but as this serves no useful purpose, it is not used in the present paper.

BOTTLE 1. Four specimens from a pony. These were all about the same size, viz., 7 mm. long and 5 mm. broad, and none contained eggs.

BOTTLE 2. Three specimens from a pony. Two of these were about 7.9 mm. long by 5.4 mm. broad, and both had eggs in their uteri. The third specimen was 5.7 mm. long by 4.4 mm. broad, and had no eggs.

BOTTLE 3. Three specimens from a horse. All three were very small, the largest being only 2.9 mm. long by 1.3 mm. broad.

BOTTLE 4. One specimen from a mule. About medium size.

BOTTLE 5. Three specimens from a horse. They were all small and averaged 4.3 mm. long by 2 mm. broad, and were all obviously immature.

BOTTLE 6. Sixty-four specimens from a horse. The maximum length of any of these worms was 4.8 mm. and the maximum breadth 2.5 mm., the minimum length was 3.2 mm. and the minimum breadth 1.3 mm., with all gradations between these two dimensions. But the longest worm was only 2.1 mm. broad, and the broadest worm was only 4.7 mm. long, therefore, the proportion of length to breadth is not absolute. The average of the whole sixty-four specimens was 4.4 mm. long by 1.9 mm. broad.

BOTTLE 7. Thirty-five specimens from a horse. These worms varied between 8.7 mm. long by 5.7 mm. broad and 5.4 mm. long by 3.2 mm. broad, and several of the larger specimens contained eggs.

(Bottles 6 and 7 were kindly lent by Mr. A. W. N. Pillers, F.R.C.V.S.).

For convenience, the points in which the two worms *P. stanleyi* and *P. collinsi* differ, according to Stiles and Goldberger (1910), are arranged in tabular form (Table VII).

TABLE VII.

	<i>P. collinsi</i>	<i>P. stanleyi</i>
1. Oesophageal portion of sucker.	Relatively broad. A dorsal and ventral 'transverse projecting ridge present.'	Relatively narrow. A dorsal ridge only present.
2. Testes.	Actually and relatively smaller.	Actually and relatively larger.
3. Opening of Laurer's canal.	A little above the posterior border of the acetabulum.	A little behind the anterior border of the acetabulum.
4. Size of acetabulum.	Relatively larger.	Relatively smaller.
5. Position of ovary and shell gland	About level of upper margin of acetabulum.	Distinctly further forward and nearer testes.

It must be noted, however, that Stiles and Goldberger record that they had only a few specimens of each of the two worms and, as they state that no eggs were seen, it is doubtful whether their worms were fully developed.

On looking through the Liverpool material, a doubt was raised in one's mind whether the differences described by Stiles and Goldberger were really of specific value, as it appears from our material that all intermediate stages between the two extremes were to be seen. With a view to examining this more fully, Table VIII was compiled from data obtained from Stiles and Goldberger (1910), from measurements of their drawings, and also from those of nine specimens taken from bottles 1, 2, 3, 6 and 7 of the Liverpool material, as far as possible representing all sizes of the worms.

Comparison of the dimensions given in the table makes it clear that Stiles and Goldberger's differences referring to the relative size of the oesophageal portion of the sucker, and the relative size of testes and acetabulum, are merely due to the stage of development of the various specimens that they examined. In all trematodes the digestive and fixation organs develop earlier than the sexual glands; therefore, it is to be anticipated that the former are relatively larger and the latter relatively smaller in young worms than in older specimens. The differences of position of the ovaries and openings of Laurer's canal are very minute, and the above authors had so little material available, that to give specific value to slight variations in positions of these organs, as they have done, does not appear to be justified.

*Historical.* Cobbold (1875a) examined thirty-three specimens of this worm from a horse in India, which were sent him by Collins, and he named them *Amphistoma collinsi* because they were smaller than a similar fluke of the elephant (*A. hawkesii*) from the same locality. A few days after publication of this paper he received from Professor Simonds another bottle of flukes from the horse, collected by Stanley in India, and in a paper (Cobbold, 1875b) he named them *Amphistoma stanleyi*, but in doing so, says 'This is apparently nothing more than a large variety of the above?' He made no detailed study of the worms in either case, and no adequate description of the internal anatomy was published until Stiles and Goldberger (1910) examined some of Cobbold's original material. The number of individuals they had for study was not large, therefore they were led to making specific characters of differences which are really due to differences in age of the specimens they examined.

*Pseudodiscus stanleyi* (Cobb., 1875) is therefore only an immature form of *Pseudodiscus collinsi* (Cobb., 1875) and must be regarded as synonymous with the latter.

TABLE VIII

Worm	Length	Breadth	Ratio of length to breadth	Ratio of diameter of oesophageal part of sucker to diameter of worm	TESTES			ACETABULUM			Distance of ovary and shell gland in front of posterior sucker	Condition of uterus
					Size	Ratio of size to distance between	Ratio of size to size of worm	Total diameter	Diameter of aperture	Ratio of diameter to length of worm		
{ From Stiles and Goldberger, 1910	<i>P. stanleyi</i> ...	5.5 to 5.6	1 : 1.82	...	...	...	...	1.7	1 to 1.25	1 : 5	...	No eggs.
	<i>P. collinsi</i> ...	3.5 to 4	1 : 1.46	...	...	...	...	1.58 by 1.1	0.5 by 0.7	1 : 3.9	...	No eggs.
	Drawing of <i>P. stanleyi</i> ...	...	1 : 1.54	1 : 1.2	...	1 : 0.4	1 : 4.8	...	...	1 : 4.5	...	
	Drawing of <i>P. collinsi</i> ...	...	1 : 1.27	1 : 7	...	1 : 2	1 : 7.0	...	...	1 : 4.1	...	
{ Author's material	Spec. 1 ...	1.4	1 : 2.2	1 : 5.4	0.059	1 : 6.3	1 : 23.6	0.572	0.416	1 : 5	0.05	Rudimentary.
	Spec. 2 ...	2.3	1 : 1.9	1 : 7.3	0.178	1 : 2.9	1 : 13	0.833	0.468	1 : 5.4	0.26	Rudimentary.
	Spec. 3 ...	3.23	1 : 1.6	1 : 10	0.468	1 : 1.3	1 : 7	1.145	0.781	1 : 4.7	0.078	Rudimentary.
	Spec. 4 ...	4.3	1 : 1.3	1 : 11	0.39	1 : 2.6	1 : 11	1.8	0.937	1 : 3.1	Touching	Partly developed.
	Spec. 5 ...	5.2	1 : 1.3	1 : 14.3	0.989	1 : 0.57	1 : 5.2	1.66	1.04	1 : 3.6	0.156	Uterus with eggs.
	Spec. 6 ...	4.16	1 : 1.3	1 : 13	0.572	1 : 1	1 : 7	1.56	0.885	1 : 4.3	0.260	Well developed; no eggs.
	Spec. 7 ...	5.36	1 : 1.26	1 : 14	1.04	1 : 0.5	1 : 7.5	1.822	1.09	1 : 4.4	0.208	Few eggs in posterior part.
	Spec. 8 ...	5.9	1 : 1.3	1 : 17.5	1.04	1 : 0.55	1 : 5.6	1.8	1.09	1 : 4.3	0.31	Well developed; no eggs.
	Spec. 9 ...	5.7	1 : 1.5	1 : 18.3	0.885	1 : 0.64	1 : 6.4	1.875	1.145	1 : 4.7	0.26	Well developed; no eggs.



*Pseudodiscus hawkesii* (Cobbold, 1875), Sonsino, 1895.

In a list of Trematodes from the Asiatic elephant, Railliet, Henry and Bauche (1914b) give the two following species of Amphistomes :—

(a) *Pseudodiscus hawkesi* (Cobbold, 1875).

SYNONYMY :—

*Amphistoma hawkesii*, Cobbold, 1875, non Piana and Stazzi, 1900.

*Pseudodiscus hawkesi*, Sonsino, 1895.

(b) *Watsonius ornatus* (Cobbold, 1882).

SYNONYMY :—

*Amphistoma ornatum*, Cobbold, 1882.

*Pseudodiscus ornatus*, Sonsino, 1895.

*Amphistoma hawkesi*, Piana and Stazzi, 1900.

*Hawkesius hawkesi*, Stiles and Goldberger, 1910.

*Watsonius ornatus*, Railliet and Henry, 1912.

Stiles and Goldberger (1910) state that their species *Pseudodiscus* (*Hawkesius*) *hawkesii* is a synonym of *Amphistoma hawkesii*, Cobbold, 1875. It therefore seems clear that *Pseudodiscus hawkesi* (Cobbold, 1875), and *Watsonius ornatus* (Cobbold, 1882) are the same. As Cobbold's original spelling was *hawkesii* and not *hawkesi* the correct name of the worm is *Pseudodiscus hawkesii* (Cobbold, 1875). This worm is easily distinguished from *P. collinsi*, because the testes in *P. hawkesii* are tandem and in *P. collinsi* they are placed side by side.

*Pseudodiscus watsoni* (Conyngham, 1904).

SYNONYMY :—

*Amphistoma watsoni*, Conyngham, 1904.

*Cladorchis watsoni*, Shipley, 1905.

*Gastrodiscus watsoni*, Verdun, 1907.

*Paramphistomum watsoni*, Manson, 1908.

*Watsonius watsoni*, Stiles and Goldberger, 1910.

*Watsonius macaci*, Kobayashi, 1915.

This species has been found on two occasions, once in man by Watson in 1904, and once in a monkey, *Cercopithecus callitrichus*, by Joyeux in 1912.



Comparison of Stiles and Goldberger's description of *P. hawkesii* and *P. watsoni* show that the two worms are practically identical. The size is somewhat different, however, *P. hawkesii* being 3.5 to 5 mm. in length by 2 mm. to 3 mm. in breadth, and *P. watsoni* 8 mm. to 10 mm. in length by 4 mm. to 5 mm. in breadth; but in *P. hawkesii* no eggs were seen, whereas in the single specimen of *P. watsoni* which these observers examined eggs were present. The difference in size is therefore probably due to difference in age. The writer is of the opinion that the two species are identical, but in view of the facts that he has not been enabled to examine either, and that one worm comes from man and the other from the elephant, he does not feel justified in merging them.

Kobayashi (1915) recorded *Watsonius macaci* from *Macacus cynomolgus* and the same author (1920) gives a description of the worm, adding a footnote to the effect that it is probably identical with the fluke identified as *W. watsoni* by Railliet, Henry and Joyeux (1912). If this is correct, *W. macaci* is a synonym of *P. watsoni*.

#### Genus *Balanorchis*, Fiscoeder, 1901.

*Definition.*—*Cladorchinae*: testes not lobed or branched; cirrus sac present and protrusible; genital sucker present.

Type species *Balanorchis anastrophus*, Fiscoeder, 1901.

Host:—*Cervidae* sp. Location:—First stomach. Locality:—Brazil.

Only one species described.

#### Genus *Pfenderius*, Stiles and Goldberger, 1910.

*Definition.*—*Cladorchinae*: testes lobed; cirrus sac present; genital sucker absent.

Type species *Pfenderius papillatus* (Cobbold, 1882), Stiles and Goldberger, 1910.

Host:—*Elephas indicus*. Location:—Colon. Locality:—India.

Only one species described.

#### Genus *Chiorchis*, Fiscoeder, 1901.

*Definition.*—*Cladorchinae*: with testes each consisting of four branches arranged like a cross; genital sucker absent and cirrus pouch present; vitellaria in two narrow rows along outer side of caeca, each follicle group

has a duct joining direct with the main longitudinal duct ; uterus as in *Paramphistomum*.

Type species *Chiorchis fabaceus* (Diesing, 1838), Fiscoeder, 1901.

Host :—Marine mammals. Location :—small and large intestine.

Locality :—?

Only one species described.

*Taxorchis*\* (Fiscoeder, 1901), gen. nov.

*Definition*.—*Cladorchinae* : testes branched ; cirrus pouch present ; genital sucker present ; main portion of uterus posterior to testes, terminal portion runs anteriorly ventral to testes.†

Type species *Taxorchis schistocotyle*, Fiscoeder, 1901.

Host :—*Dictocotyle torquatus*. Location :—Caecum. Locality :—Brazil.

Only one species described.

Genus *Cladorchis*,‡ Fiscoeder, 1901.

*Definition*.—*Cladorchinae* : with branched testes, genital sucker usually present ; cirrus sac present ; uterus as in the genus *Paramphistomum*.

Type species *Cladorchis pyriformis* (Diesing, 1838), Fiscoeder, 1901.

KEY TO SPECIES

Testes in tandem	...	...	...	...	...	...	...	...	...	1
Testes side by side	...	...	...	...	...	...	...	...	...	2
1. Posterior sucker on ventral surface at junction of posterior and middle thirds, small and contains no papillae										
...										
<i>C. pyriformis</i> (Diesing, 1838), Fiscoeder, 1901.										
Host :— <i>Tapirus americanus</i> , S. America										

\* Fiscoeder classified the species *T. schistocotyle* under the genus *Cladorchis*, sub-genus *Taxorchis*. But the course of the uterus is so different from any other member of the genus that it is considered advisable to place this species in a separate genus.

† The uterus arises from the shell gland just in front of the posterior sucker ; from this point it runs forward near the dorsal surface to the posterior border of the testes, it then turns posteriorly and runs diagonally towards the ventral surface to just in front of the posterior sucker ; after reaching this point it again turns forward and runs close to the ventral surface to the genital pore.

‡ Fiscoeder divided this genus into three sub-genera, viz., *Cladorchis*, *Stichorchis*, and *Taxorchis*. The last has already been dealt with, having been raised to generic rank. *Cladorchis* and *Stichorchis* do not present striking anatomical differences, so they will be considered together, the sub-generic titles being dropped.

Posterior sucker occupies hinder half of ventral surface; large (2 mm.-4 mm.) and beset with papillae with special structure ... ..

*C. asper*, Fiscoeder, 1901.  
Host :—*Tapirus americanus*,  
S. America

2. Pharyngeal pouches projecting from sucker; genital sucker distinct ...

*C. giganteus* (Diesing, 1838),  
Fiscoeder, 1901.  
Host :—*Dicotyles* spp.

Pharyngeal pouches so small that they do not show on external wall of sucker; genital sucker not distinct ... ..

*C. subtriquetrus* (Rudolphi, 1814),  
Fiscoeder, 1901.  
Host :—*Castor fiber*, *Bos taurus*.

Sub-family *STEPHANOPHARYNGINAE*, Stiles and Goldberger, 1910.

*Definition*.—*Paramphistomidae*: with a single oral diverticulum.

Only one genus.

Genus *Stephanopharynx*, Fiscoeder, 1901.

*Definition*.—That of the sub-family.

Type species *Stephanopharynx compactus*, Fiscoeder, 1901.

Only one species recorded.

*Stephanopharynx compactus*, Fiscoeder, 1901.

Fiscoeder found the worm in *Bos* sp.

The material available to the writer consisted of the following collections :—

1. About 850 specimens.
2. Over one hundred specimens.
3. Ten specimens.

These collections were found in the stomachs of three waterbuck (*Cobus* sp.) at Ngoa, N.E. Rhodesia.

Fiscoeder's material consisted of two collections composed of a single specimen in one case and two specimens in the other; they were found in company with many other *Amphistomata*.

In addition to the single large oral diverticulum, this worm is characterised by the anterior end being hemispherical instead of the usual conical type, and its maximum transverse diameter is about its middle instead of

towards the posterior end which is the ordinary condition in *Paramphistomidae*. The posterior sucker looks ventrally on account of the strong downward curve which the hinder half of the body exhibits, and it is said to have a surprisingly sharp border around its opening. The testes lie one behind the other, the anterior one being about the middle of the body, with the hinder one between it and the posterior sucker. Laurer's canal opens in front of the excretory pore in the mid-line of the dorsal surface, and the genital pore is surrounded by a thickening of the subcuticular muscle not sharply marked off from the parenchyma. The size of these worms is given as 4.8 to 5 mm. in length, 3 mm. in breadth, and 2.5 mm. in thickness. Other anatomical details are of the usual type. The writer found in his collection No. 1 eight worms, and in his collection No. 2 one worm which agreed with Fiscoeder's description in all particulars, except that they were about 7 mm. in length. The remaining numerous specimens, however, at first sight appeared to be very different, as they were sharply pointed at the anterior end and the posterior sucker looked directly backwards, in many cases being widely opened and occupying the whole hinder end of the worm. (Plate VII, fig. B). These worms were of all sizes from tiny specimens about 2 mm. in length up to worms 5.5 mm. in length. Several of these worms were sectioned and it was then found that they agreed with Fiscoeder's species, except that the posterior sucker was relatively larger, and the hinder of the two testes was much nearer the ventral surface than the anterior one (fig. 12). At first it was thought that these points indicated a new species, but it was then noted that none of the worms were gravid, and prolonged search of the ample material failed to reveal any specimen containing eggs. On this account it is considered that they are immature specimens of *S. compactus*, which fact explains the relatively large size of the posterior sucker. The apparently different position of the testes in young worms is not without parallel, for Fiscoeder says that in immature *P. calicophorum*, the anterior testis lies more dorsal than the hinder one, a character which the writer has found disappears when maturity is reached. A further fact in support of the view that the present worms are only immature *S. compactus* is that this species has only been recorded on five occasions (twice by Fiscoeder and three times by the writer) and on two of these it has been found in company with worms of the above closely allied type.



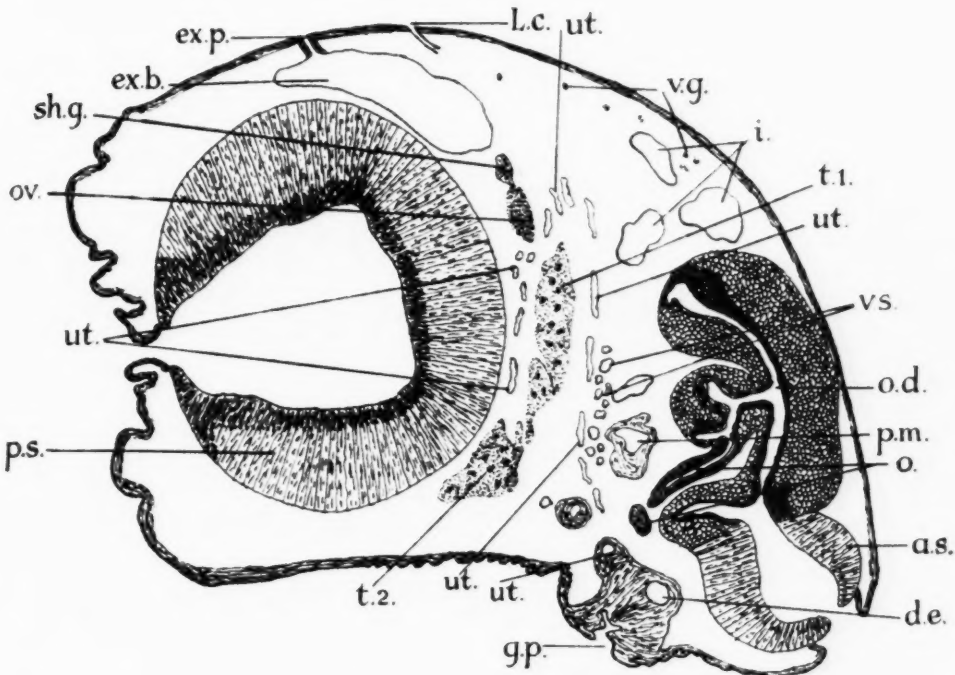


FIG. 12. *Stephanopharynx compactus*. Sagittal section near the mid-line. *a.s.*—anterior sucker; *d.e.*—ductus ejaculatorius; *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *g.p.*—genital pore; *i.*—intestine; *L.c.*—Laurer's canal; *o.*—oesophagus; *o.d.*—oral diverticulum; *ov.*—ovary; *p.m.*—pars muscosa; *p.s.*—posterior sucker; *sh.g.*—shell gland; *n.*—dorsal testis; *v.*—ventral testis; *ut.*—uterus; *vi.g.*—vitelline gland; *vi.s.*—vesicula seminalis.  $\times 14$ .

Family *GASTROTHYLACIDAE*, Stiles and Goldberger, 1910.

*Definition.*—*Amphistomata*: with a ventral pouch.

## KEY TO GENERA

- |    |   |        |                       |
|----|---|--------|-----------------------|
| 1. | Uterus crosses from one side of body to the other near the middle of the worm | ... .. | <i>Gastrothylax</i>   |
|    | Uterus lies in centre of body for its whole length                            | ... .. | 2                     |
| 2. | Testes side by side   | ... .. | <i>Carmyerius</i>     |
|    | One testis dorsal of the other, both in mid-line                              | ... .. | <i>Fischboederius</i> |

Genus *Gastrothylax*, Poirier, 1883.

Fischoeder (1903) placed all the known *Paramphistomidae* with a ventral pouch in the genus *Gastrothylax*. He divided the species in this genus into five groups, using the shape of the ventral pouch on cross section as the distinguishing feature. His list is as follows :—

Genus *Gastrothylax*.

(a) Transverse section of pouch triangular with apex dorsally directed and apical angle undivided.

1. *G. crumenifer* (Crepl.).
2. *G. compressus*, Brandes.



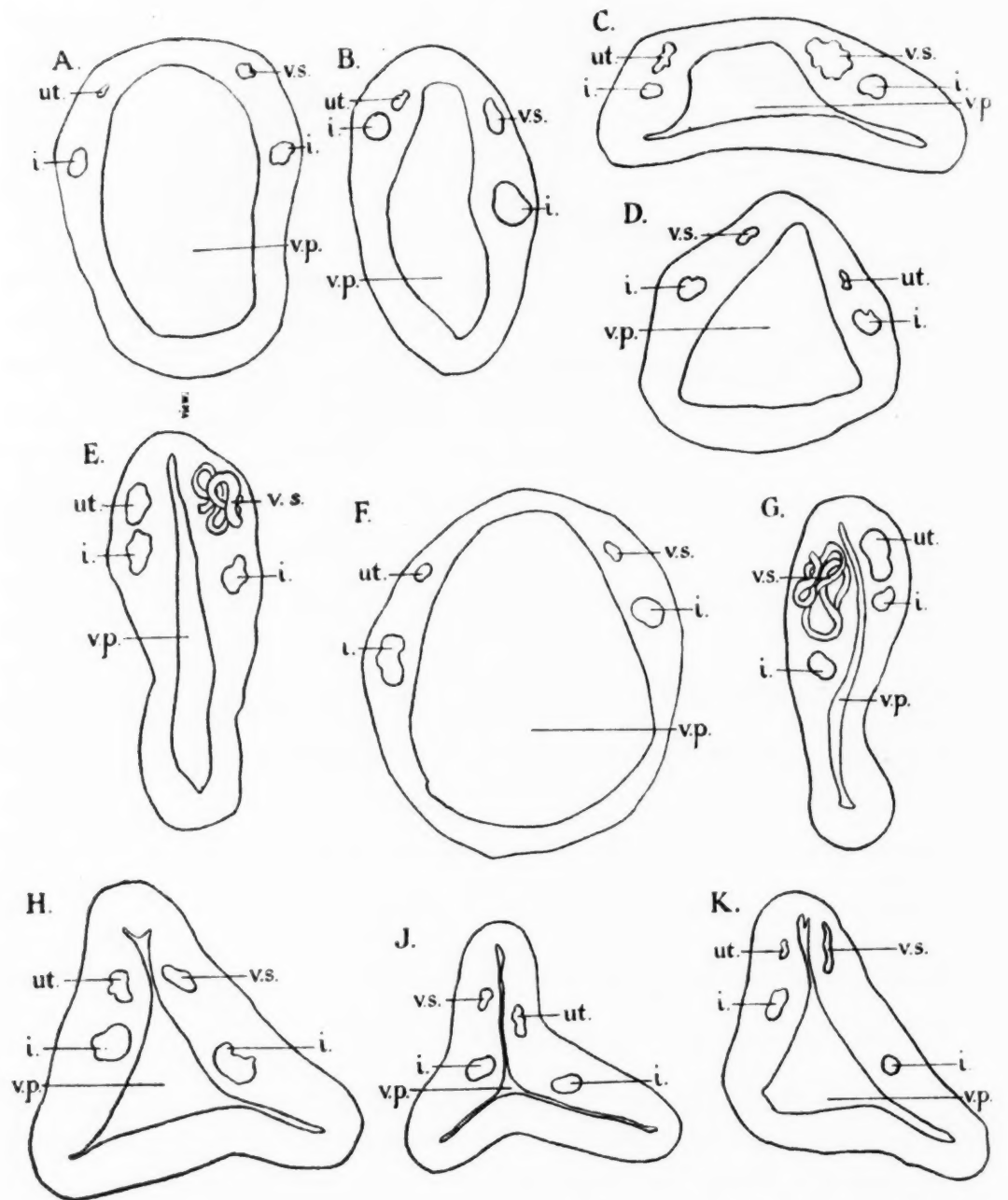


FIG. 13. *Gastrobylax crumenifer*. Transverse sections through the middle of ten specimens showing variation in shape of ventral pouch. *i.*—intestine; *ut.*—uterus; *vp.*—ventral pouch; *v.s.*—vesicula seminalis.  $\times 9$ .

(b) Transverse section of pouch triangular with apex dorsally directed and apical angle bifurcated.

3. *G. gregarius*, Looss.

(c) Transverse section of pouch circular.

4. *G. spatiosus*, Brandes.

(d) Transverse section of pouch triangular with apex ventrally directed.

5. *G. synethes*, Fischdr.

6. *G. elongatus*, Poirier.

7. *G. cobboldi*, Poirier.

8. *G. mancupatus*, Fischdr.

(e) Transverse section of pouch triangular with apex ventrally directed. The two basal angles bifurcated.

9. *G. minutus*, Fischdr.

The writer has carefully investigated the shape of the ventral pouch in cross section in all the species he has had at his disposal. Fig. 13 illustrates transverse sections at the mid point of ten specimens of *G. crumenifer*. All the specimens agreed in other anatomical details and all came from the same bottle. Fig. 14 represents similar sections of six specimens of *G. spatiosus*.

On examining the drawings it will be seen that fig. 13, D, corresponds with Fiscoeder's group (a), fig. 13, H and K, correspond with his group (b), and fig. 13, F, is so nearly circular as to correspond with his group (e). Fig. 13, A, B, C, E and J, do not agree with any of Fiscoeder's five groups. Therefore, in a series of ten specimens of a single species three of Fiscoeder's five groups are represented, and the others cannot be classified by this method. If fig. 13, D, H and K, are further examined, it will be noted that they are all triangular with the apex dorsal, whereas in fig. 14, B and D, which approach the triangular shape, the apex is ventral. This at first seems to indicate that a division between the species might be made on the fact that when the pouch is triangular, the apex is ventral in some species and dorsal in others. But fig. 14, A, upsets this view, because here is a worm considerably distorted in fixation with the dorsal surface drawn to the right and the ventral surface displaced to the left. The ventral pouch in this case is a narrow triangle with the base to the right. If this worm could be straightened up, one would have a specimen with the base

of the ventral pouch looking towards the ventral surface, unlike the other examples of the same species in which the base of the pouch, when triangular, is dorsally directed. From this it will be seen that the cross section of the ventral pouch may assume almost any shape and cannot therefore be regarded as of any use in diagnosis.

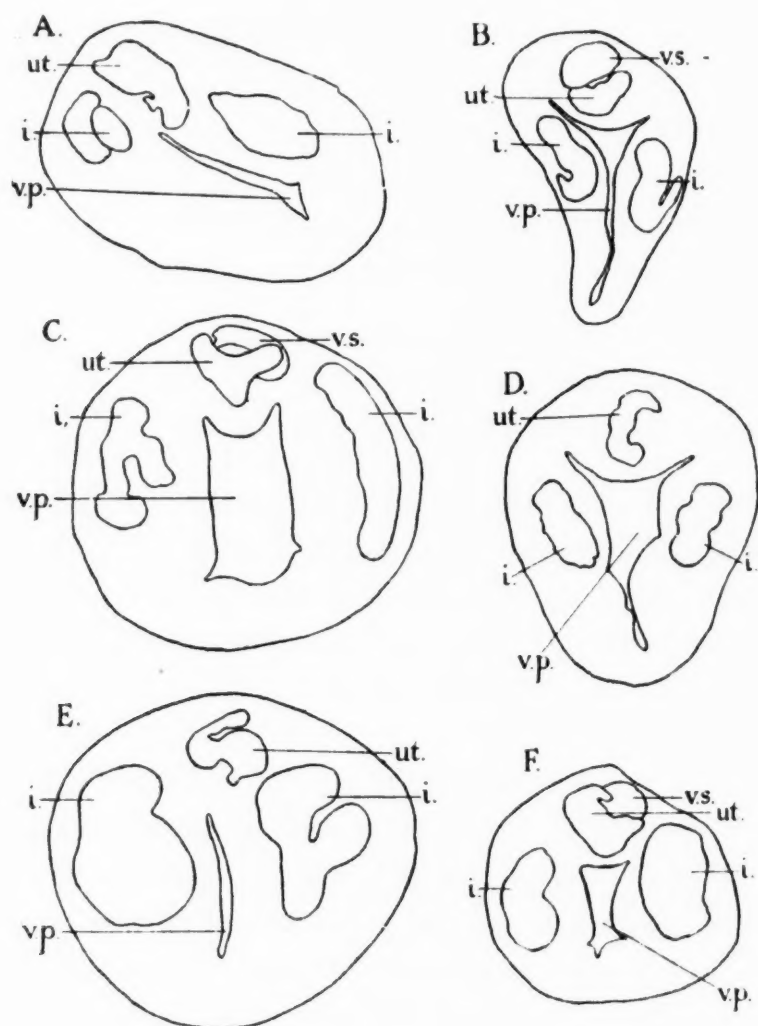


FIG. 14. *Carmyrius spatiosus*. Transverse sections through the middle of six specimens showing variations in shape of ventral pouch. *i.*—intestine; *ut.*—uterus; *vp.*—ventral pouch; *vs.*—vesicula seminalis.  $\times 9$ .

The shape of the ventral pouch when seen from the side also varies considerably, as reference to figs. 15, 16, and 17 will readily show. For the most part the changes in shape of the pouch when viewed in this position are caused by outpocketings from the posterior end along the dorsal and ventral surfaces of the testes, but in a few cases a narrow prolongation may also occur from the dorso-anterior part of the pouch (see fig. 17, C). In addition to differing as a whole, the pouch also exhibits varying form in

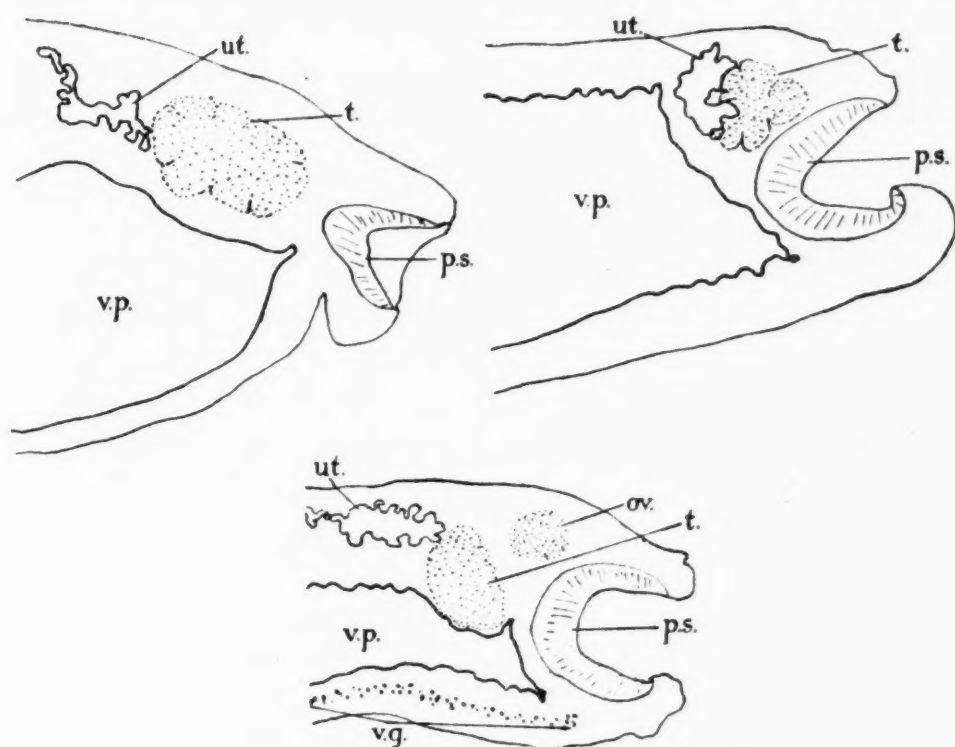


FIG. 15. *Gastrothylax crumenifer*. Sagittal sections near the mid-line of three specimens to show the shape of the posterior end of the ventral pouch. *ov.*—ovary; *p.s.*—posterior sucker; *t.*—testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch.  $\times 9$ .

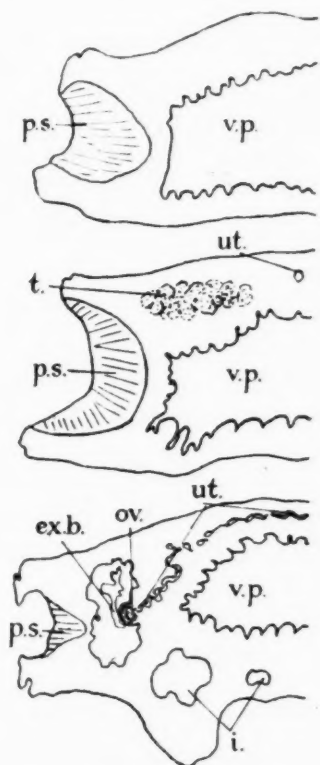


FIG. 16. *Gastrothylax crumenifer*. Three sagittal sections from a single specimen at different levels, showing change in shape of posterior end of pouch in one worm. *ex.b.*—excretory bladder; *i.*—intestine; *ov.*—ovary; *p.s.*—posterior sucker; *ut.*—uterus; *v.p.*—ventral pouch.  $\times 9$ .

different sections of the same worm (fig. 16). It will be noted later that Stiles and Goldberger use the presence of a ventral prolongation of the pouch beneath the testes as a specific character in at least one instance.

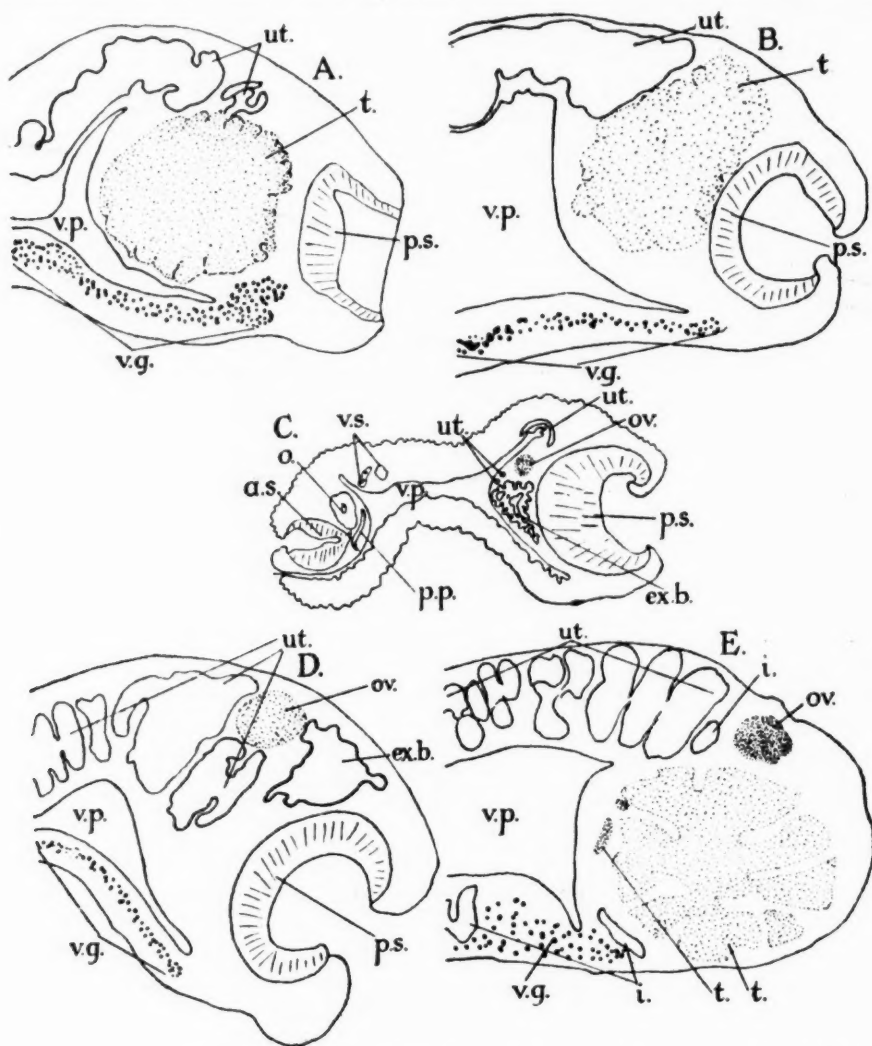


FIG. 17. *Carmyrius spatiosus*. . Sagittal sections of five specimens near the mid-line showing variation in shape of posterior end of pouch. A, B, D, and E—Gravid worms. C—Immature worm. a.s.—anterior sucker; ex.b.—excretory bladder; i.—intestine; o.—oesophagus; ov.—ovary; p.p.—pars prostatica; p.s.—posterior sucker; t.—testis; ut.—uterus; v.g.—vitelline gland; v.p.—ventral pouch; v.s.—vesicula seminalis.  $\times 9$ .

The above demonstration of the presence or absence of this prolongation in a single species (fig. 17) is held to prove that this character is of no value for specific diagnosis.

Genus *Gastrothylax* (Poirier, 1883), s. str., Stiles and Goldberger, 1910.

*Definition.*—*Gastrothylacidae*: with the uterus crossing from one side of the body to the other about its middle.

Type species *Gastrothylax crumenifer* (Creplin, 1847).

Only one species recorded.



*Gastrothylax crumenifer* (Creplin, 1847).

SYNONYMY:—*Gastrothylax compressus*, Brandes, 1898.

Stiles and Goldberger (1910) revised Fiscoeder's classification and restricted the genus *Gastrothylax* to include only those species in which the uterus crosses from one side of the worm to the other about midway between the anterior and posterior extremities. The result of this restriction is that the genus now contains only two species, viz., *G. crumenifer* and *G. compressus*. The writer has not been able to consult the original descriptions of these species, but Fiscoeder (1903) describes them very fully and use has been made of this work in the present discussion.

The points of difference between these two worms are summarised from Fiscoeder (1903) in Table IX.

TABLE IX.

Points of difference between *G. compressus* and *G. crumenifer*.

	<i>G. compressus</i>	<i>G. crumenifer</i>
Length of oesophagus ...	400 $\mu$ to 500 $\mu$	1.2 mm. to 1.5 mm.
Gut caeca ...	Almost straight. End 300 $\mu$ to 500 $\mu$ anterior to testes.	Wavy. End behind anterior border of testes.
Uterus ...	Not so convoluted.	More convoluted.
Eggs ...	Not so numerous. 115 $\mu$ to 125 $\mu$ by 60 $\mu$ to 65 $\mu$ .	More numerous. 125 $\mu$ to 135 $\mu$ by 65 $\mu$ to 70 $\mu$ .

In the present instance the material available for examination consisted of two bottles containing several hundred specimens. One collection came from a bullock in India and the other from a bullock in Hong Kong. Examination of a large number of these worms has shown that like all other *Amphistomata*, they are liable to considerable variation. In our collections there are many worms which could be identified as one or other of the above species, and there are just as many which would fit either species just as well. It is therefore considered that these two species have been separated from one another on points of individual variation, and that the differences are so variable in degree that in all probability *G. compressus* is merely a synonym of *G. crumenifer*.

Genus *Fischoederius*, Stiles and Goldberger, 1910.

*Definition.*—*Gastrothylacidae* : with the uterus in the centre of the body for its whole length ; one testis dorsal of the other.

Type species *Fischoederius elongatus* (Poirier, 1883), Stiles and Goldberger, 1910.

## KEY TO SPECIES

Gut caeca extend at least to testes ... ..	<i>F. cobboldi</i>
Gut caeca never extend further than just beyond middle of body	<i>F. elongatus</i>

*Fischoederius elongatus* (Poirier, 1883), Stiles and Goldberger, 1910.

## SYNONYMY :—

*Fischoederius fischoederi*, Stiles and Goldberger, 1910.

*Fischoederius siamensis*, Stiles and Goldberger, 1910.

*Fischoederius ceylonensis*, Stiles and Goldberger, 1910.

First found in the stomach of *Palonia frontalis* in Java.

The material available to the writer consisted of about 25 specimens obtained from a bullock in Hong Kong.

Eight specimens were cleared in carbolic acid and their examination showed that the worm closely agrees with Fischoeder's (1903) description, with the proviso that this author does not take cognisance of individual variations. For instance, Fischoeder states that the caeca end within the beginning of the hinder half of the body, but in the eight specimens examined, this was found to be the case only in three of them ; in one the caeca ended exactly at the junction of the anterior and posterior halves of the body, and in the remaining four specimens they ended at varying distances up to as much as 1 mm. in front of it. In three cases the two caeca were of unequal length ; in the most marked instance the gut on one side was 1 mm. longer than on the other. In some of the worms examined, the caeca were as convoluted as Fischoeder shows in his fig. 59, but in others they were nearly straight, and furthermore some caeca presented short dilated portions in various parts of their course, while in others they were of uniform diameter throughout.

Fischoeder also says that the testes lie one above the other near the mid-line, the ventral one being slightly to the right of the mid-line and slightly posterior to the dorsal, which is rather to the left of the mid-line. This was true of some of the specimens examined, but in others one testis appeared to lie directly above the other, and both of them were exactly in the mid-line.

*Fischoederius fischoederi*, Stiles and Goldberger, 1910.

This species is stated to have been made from a single specimen found in a bottle containing a number of worms which had been determined by Fischoeder as *G. elongatus* (*F. elongatus*). They distinguished it from the latter on the ground that the gut caeca were very slightly longer, and that the ovary and shell gland lie between the testes in *F. fischoederi*, which they state is not the case in *F. elongatus*. With regard to the position of the ovary in *F. elongatus*, Fischoeder (1903) states:

‘Der fast kuglige Keimstock (0.3 bis 0.35 mm. im Durchmesser) liegt dorsal von der Schalendrüse, etwas seitlich von der Medianlinie dicht hinter und etwas median von dem meist nach hinten herabhängenden dorsalen Ende des vordern Hodens (Text fig. L).’

It will be noted that he makes no reference to the other testis, but Text fig. L, to which he refers, shows the ovary and shell gland lying between the two testes, but a little further back than Stiles and Goldberger show them in fig. 2. As it is recognised that the ovary varies slightly in position in most species, this difference cannot be considered of specific importance, especially as it relates to an isolated specimen taken from a collection of *F. elongatus* which had already been determined by Fischoeder. It is therefore considered that *F. fischoederi* is a synonym of *F. elongatus*.

*Fischoederius siamensis* and *F. ceylonensis*, the other two new species made by Stiles and Goldberger, are separated from *F. elongatus* because they do not exhibit the prominent bulging round the genital pore which these authors state is present in *F. elongatus*. It is true Fischoeder (1903) in his description of *F. elongatus* mentions this bulging, but from the experience of the writer, it is concluded that the bulging is a variable character and is similar to that which may be found in any species of the group *Amphistomata* and is accordingly of no specific value. It is therefore considered that *F. siamensis* is synonymous with *F. elongatus*.

*F. ceylonensis* was established as a new species on a single specimen taken from a bottle of *G. synethes*. Stiles and Goldberger distinguish between *F. ceylonensis* and *F. siamensis* by two points; firstly, the testes in *F. ceylonensis* are directly one above the other and in *F. siamensis* they are slightly diagonal; and secondly, the ventral pouch in *F. ceylonensis* extends ventral to the testes, whereas in *F. siamensis* this extension of the ventral pouch does not occur. The individual variations which may be found in the ventral pouch have, however, already been fully discussed,

and it is considered that the differences between *F. ceylonensis* and *F. siamensis* are not of specific value, more especially as the diagnosis of one of them rests on a single specimen.

*Fischoederius cobboldi* (Poirier, 1883), Stiles and Goldberger, 1910.

First found in the stomach of *Palonia frontalis* in Java.

No material of this species was available to the writer.

From Fischoeder's description, the distinguishing feature between *F. cobboldi* and *F. elongatus* is the difference in position of the termination of the caeca. In *F. cobboldi* they end opposite the base of the sucker, that is posterior to the testes and near the hinder end of the worm, and in *F. elongatus* they end about the middle of the worm. Although it has been shown that the point of termination of the caeca is subject to variations in practically all species of the group, in the present instance the differences between the two species under discussion are so marked that it is unlikely that they can be explained in this manner. It is accordingly considered that *F. cobboldi* and *F. elongatus* can be distinguished by the difference in length of the caeca.

Genus *Carmyerius*, Stiles and Goldberger, 1910.

SYNONYMY :—*Wellmanius*, Stiles and Goldberger, 1910.

*Definition*.—*Gastrothylacidae* : with the uterus in the centre of the worm for its whole length ; testes side by side.

Type species *Carmyerius gregarius* (Looss, 1896), Stiles and Goldberger, 1910.

#### KEY TO SPECIES

- |    |  |     |     |     |                       |
|----|--|-----|-----|-----|-----------------------|
| 1. | Genital pore lies outside ventral pouch                              | ... | ... | ... | <i>C. exoporus</i>    |
|    | Genital pore lies within ventral pouch                               | ... | ... | ... | 2                     |
| 2. | Excretory canal and Laurer's canal unite before reaching the surface | ... | ... | ... | <i>C. wenyoni</i>     |
|    | Excretory canal and Laurer's canal do not unite                      | ... | ... | ... | 3                     |
| 3. | Cross section of ventral pouch shows five angles                     | ... | ... | ... | <i>C. cruciformis</i> |
|    | Cross section of ventral pouch does not show five angles             | ... | ... | ... | 4                     |
| 4. | Gut caeca extend to testes   | ... | ... | ... | <i>C. spatiosus</i>   |
|    | Gut caeca do not extend beyond middle of worm                        | ... | ... | ... | <i>C. gregarius</i>   |

Stiles and Goldberger made this genus to include all the species of Poirier's *Gastrothylax* in which the uterus occupies a central position for its whole length, the testes lie side by side, and the vas deferens is without a straight portion at its commencement. This included the following five



species: *C. synethes* (Fischoeder, 1901), *C. gregarius* (Looss, 1896), *C. spatiosus* (Brandes, 1898), *C. mancupatus* (Fischoeder, 1901), and *C. minutus* (Fischoeder, 1901). They divide the above five species into two groups, because the first two worms are said to have a genital atrium with a large ventral chamber and the last three a genital atrium without a ventral chamber. These characters have been fully discussed already and it has been shown that presence or absence of chambers in the atrium, or even the existence of the atrium itself, are purely matters of chance, so that it seems reasonable to assume that this point is of no value in the present instance. Differences in shape of the ventral pouch are also mentioned in the definitions of the various species. The unreliability of this character has already been dealt with.

*Carmyerius spatiosus* (Brandes, 1898).

SYNONYMY:—

*Carmyerius synethes*, Fischoeder, 1901.

*Carmyerius minutus*, Fischoeder, 1901.

*Carmyerius mancupatus*, Fischoeder, 1901.

*Gastrothylax bubalis*, Innes, 1912.

*Wellmanius wellmani*, Stiles and Goldberger, 1910.

First found in the stomach of *Bos taurus* in Arabia.

Material available:—Two collections from Ngao, N.E. Rhodesia; the host in one case was a roan (*Hippotragus equinus*) and in the other a reedbuck (*Cervicapra* sp.).

As the four species *C. spatiosus*, *C. synethes*, *C. minutus* and *C. mancupatus* only differ in minute points apart from those already dealt with, they will be discussed together.

According to Stiles and Goldberger's key, the only point by which these four species may be distinguished are minute differences in the gut caeca. They write that in *C. synethes* the caeca are 'corkscrew-like, rather narrow and long'; in *C. spatiosus* they are 'straight, narrow and rather long'; in *C. mancupatus* they are 'rather sinuous, narrow, and long'; and in *C. minutum* they are 'swollen in their caudal half and are rather long.' In all four cases the caeca are said to end in the 'fourth zone.' Twenty specimens were cleared in carbolic acid and it was found that the degree of convolution of the caeca was so variable as to be of no use for diagnosis. With regard to differences in diameter of various parts of the caeca, they were found swollen or contracted in any part of their course or of uniform



diameter throughout. Fig. 18, A and B are drawings of the two caeca in a single worm showing alternate contractions and dilatations; fig. 18, C, is a drawing from another specimen showing the gut of uniform diameter from end to end. It is therefore clear that dilatation of a special part of the caeca is of no diagnostic value.

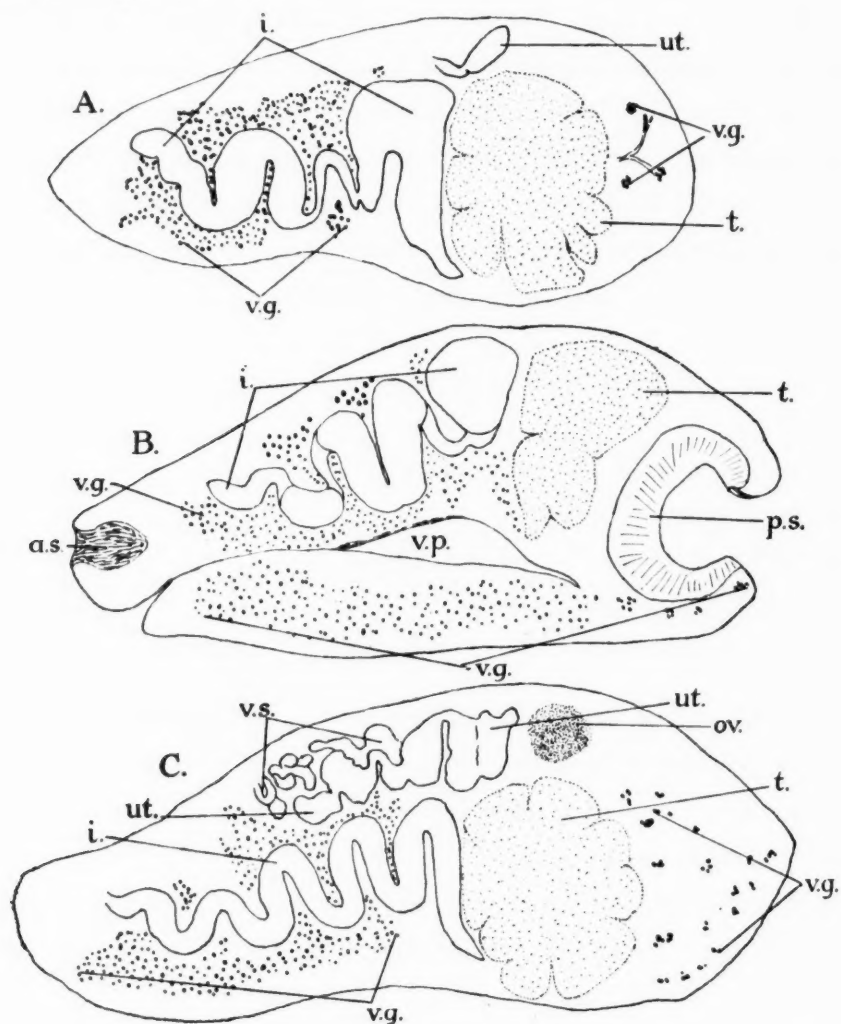


FIG. 18. *Carmyrius spatiosus*. Sagittal section to show variation in calibre of gut. A and B—Showing the two sides of the same worm. C—Section of another worm. a.s.—anterior sucker; i.—intestine; ov.—ovary; p.s.—posterior sucker; t.—testes; ut.—uterus; v.g.—vitelline gland; v.p.—ventral pouch; v.s.—vesicula seminalis.  $\times 9$ .

Fischoeder (1903) mentions one or two other small points of difference, viz., the genital pore lies further posteriorly in *C. synethes* than in any other species, being about 1 mm. behind the entrance to the ventral pouch; but as he gives 0.8 mm.-0.9 mm. and 0.7 mm.-0.8 mm. for the same dimension in *C. spatiosus* and *C. mancupatus* respectively, it cannot be considered justifiable to regard this of specific value, especially as he gives the limits of variation in length of the worms as being between 6 mm.-12 mm. for the three species.

Fischoeder states that *C. minutus* is 4 mm.-5 mm. in length ; then he remarks that some specimens are longer and thinner than this, but gives no other measurements. This point cannot be regarded of any value when he says that *C. spatiosus*, *C. synethes* and *C. mancupatus* are 9 mm.-12 mm., 7 mm.-11 mm., and 6 mm.-11 mm. in length respectively. Another point of difference claimed between *C. minutus* and the other three species, is that the ovary and shell gland are said to be more strongly developed in the former. But difference in size of the genital organs is so dependent on the age of an individual that it cannot be regarded as a satisfactory diagnostic character.

From the above observations it is concluded that Fischoeder's three species, *C. synethes*, *C. mancupatus* and *C. minutus* are synonyms of *C. spatiosus* (Brandes).

*Carmyerius wenyoni* (Leiper, 1908).

Found in the stomach of *Cobus maria* at Taufikia, White Nile.

It is not included in Stiles and Goldberger's (1910) classification. It is similar in most respects to *C. spatiosus*, but there is one point by which it may be clearly distinguished, and that is that the excretory canal and Laurer's canal unite before reaching the surface of the worm and open by a common pore. This character is very remarkable as it has not been observed in any other species.

*Carmyerius cruciformis* (Leiper, 1910).

The material available for study consisted of a single collection of over one hundred specimens from a hippopotamus killed in Lake Victoria, Nyanza.

This worm was first described by Leiper who states that he only had immature material. Leiper gives the length of this worm as from 0.5 mm. to 0.8 mm.; this is apparently a mistake for 5 mm. to 8 mm. because he gives the dorso-ventral diameter of the posterior sucker as 0.9 mm. with a muscle wall of 1.8 mm. in thickness. Some of the writer's specimens contained eggs in the uterus and these worms measured 5 mm. to 7 mm. in length with a maximum diameter of about 1.5 mm.; they agree in general anatomical details with Leiper's description. The uterus

was observed to pursue a slightly wavy course along the centre of the dorsal surface; the most heavily gravid specimen only contained about thirty eggs disposed in a single chain and not closely packed into the uterus in the usual manner. Eggs removed from one specimen measured about  $135-140\mu$  in length by  $80-84\mu$  in breadth; on account of the small size of the worm they appeared relatively large. Leiper named this worm 'cruciformis' because he says the ventral pouch always shows five angles in cross section no matter how contracted the worm may be, and this frequently appears as a cross. The writer cut eight specimens transversely, and although the pouch of three of them exhibited five angles none were at all like a cross and the other five specimens exhibited just as extensive variation as do all *Gastrothylacidae* in respect of the shape of the pouch.

This worm is essentially the same as *C. spatiosus* in details of anatomy, but is easily distinguished from this species by its minute size even in the gravid state.

*Gastrothylax bubalis*, Innes, 1912.

This worm was recorded by Innes (1912) as a new species. It was found in the stomach of the Hartebeest in Rhodesia.

The general anatomy of the worm is apparently in no way different from *C. spatiosus*; to quote from Innes the identification of the species is based on the following characters:—

'The excretory vesicle of this species is unique both in position and outline. It is situated immediately in front of the posterior sucker and behind the shell gland, while in other species it has a more or less lateral position. The great irregularity of its outline is striking.'

No authority is given for the statement that the excretory bladder lies in a more or less lateral position in other species, and Fischöeder (1903) states that in *G. spatiosus*:

'Die sehr grosse Excretionsblase liegt zwischen der Bauchtasche und dem Saugnapfe einerseits und den beiden Hoden andererseits (fig. 52c. u. 54).'

And in all other species of the genus that he deals with he describes it in a similar position, only alluding to slight alterations due to change of shape, and his figures all show it in the same position as it is seen, Innes' fig. 7. It therefore seems clear that this difference claimed by Innes does not exist. With regard to his other point, viz., the irregularity of the excretory bladder, it may be observed that irregularity of outline is

characteristic of a partly distended bladder in any species, and is obviously of no specific value. From this it is concluded that the species *G. bubalis* is only a synonym of *C. spatiosus*.

*Carmyerius gregarius* (Looss, 1896), Stiles and Goldberger, 1910.

First found in the stomach of *Bos bubalis* in Egypt.

Material available.—A single collection of about 25 specimens from a buffalo.

This species is easily distinguished from the other species of the genus, as the gut caeca are very short. Of ten specimens cleared in carbolic acid only one was found in which the caeca reached the middle of the worm; in all the others they ended at varying distances anterior to this point. For the most part the caeca were of uniform diameter throughout, but in one specimen they exhibited a swelling about their middle and in another there was a club-shaped swelling at their extremities. Although variation in the length of the caeca has been found unreliable for specific diagnosis when the range is slight, they are, however, so much shorter in *C. gregarius* than in *C. spatiosus* that this difference is considered of specific value in the present instance.

*Carmyerius exoporus*, n.sp.

Found in the stomach of a *Tragelaphus spekei* in Nyasaland.

The material available was a single collection of over three hundred specimens.

#### EXTERNAL ANATOMY

All stages of growth were represented in the collection, from worms no bigger than a raspberry seed up to gravid worms measuring over 11 mm. in length. But even in gravid worms the shape and size was subject to great variation (see Plate VII, A). It will be noted in this plate that in some cases the worms are fully extended (9) and in others they are in a state of contraction (7). In view of the great variation in shape that exists, it is not considered worth while giving a detailed description, but as a rough indication of what the size of gravid worms is, it may be stated that they varied from 11.5 mm. in length by 2.6 mm. in breadth to 5 mm. in length by 3.8 mm. in breadth. In all cases the worms were practically circular on cross section. As the worms are not curved ventrally, the opening of the anterior sucker lies at the extreme anterior end and looks directly



forwards. The opening of the ventral pouch lies in the mid-line of the ventral surface close behind and below the oral opening, and between the two is the opening of the genital pore, which is thus outside the ventral pouch. This character is sufficient to distinguish the present species from any other member of the genus *Carmyrius*, as in all the hitherto described species the genital pore opens within the ventral pouch. The posterior sucker, as a rule, looks directly backwards, but in a few cases it is slightly tilted ventrally.

#### INTERNAL ANATOMY

The general arrangement of the organs of this species is very similar to *G. spatiosus* and on that account only the briefest description is necessary.

*Muscular system.* This system exhibits no special characters.

*Nervous system.* This system was not investigated.

*Anterior sucker.* The anterior sucker is of the typical globular shape, oval in section.

*Intestines.* The oesophagus arises from the posterior end of the anterior sucker and after pursuing a dorsally curving course of about 300 $\mu$ , it divides into the two gut caeca. These two canals pursue a wavy course along each side of the worm and terminate about the level of the testes.

*Posterior sucker.* The posterior sucker is fairly thick-walled and looks directly backwards or slightly towards the ventral surface (figs. 20, 21 A, and 25).

*Excretory system.* The excretory bladder is of the usual type and its degree of convolution varies with its state of distension. The excretory canal runs dorso-posteriorly and opens in the mid-line of the dorsal surface above the anterior border of the posterior sucker (fig. 20).

*Genitalia. Testes.* The testes are large lobed organs lying one on each side of the mid-line near the hinder end of the worm (fig. 21 A).

*Vas deferens.* This duct is composed of the usual three portions, the vesicula seminalis, the pars muscosa, and the pars prostatica. The first two portions are much convoluted and occupy the anterior third of the centre of the dorsal part of the worm. The pars prostatica is relatively long and straight, and runs directly forwards to enter the genital papilla (figs. 19 and 21, B).

*Genital pore.* The genital pore is surrounded by a muscular thickening not marked off from the parenchyma and it opens in the middle of the worm between the oral opening dorsally and the opening of the ventral pouch



ventrally (figs. 19 and 22). It is of the usual type in structure and the appearance shown in the figures is not the only one met with, as the genital papilla is capable of complete retraction or extrusion as in other species. In the figures it is in the intermediate condition.

*Ovary and shell gland.* These two structures lie between the testes on one hand, the base of the ventral pouch in front and the posterior sucker on the other (figs. 20 and 21 A).

*Laurer's canal.* Laurer's canal runs almost directly dorsally from the shell gland and opens in the mid-line well in front of the excretory pore (fig. 20).

*Vitellaria.* For the most part these glands lie in the ventral portion of the worm, but they extend for varying distances on each side towards the dorsal surface. As a rule, they extend from the posterior end of the anterior sucker in front, to opposite the testes behind, but in a few cases follicles were found close to the posterior end of the worm, and surrounding the opening of the posterior sucker (figs. 19, 20, 21, A, and 25).

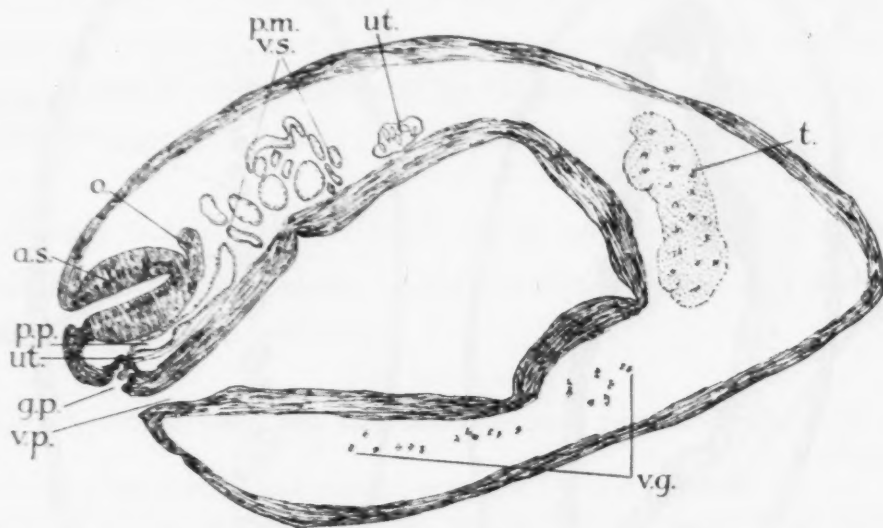


FIG. 19. *Carmyerius exoporus*, n.sp. Sagittal section. a.s.—anterior sucker; g.p.—genital pore; o.—oesophagus; p.m.—pars muscosa; p.p.—pars prostatica; t.—testis; ut.—uterus; v.g.—vitelline gland; v.p.—ventral pouch.  $\times 18$ .

*Uterus.* The uterus pursues a wavy course along the centre of the dorsal surface; anteriorly it terminates by running ventral to the pars prostatica and uniting in the genital papilla with the male duct (figs. 21 B and 22).

*Eggs.* These are oval, operculated and measure about  $115\mu$  to  $130\mu$  in length by  $60\mu$  to  $68\mu$  in breadth, but they were only taken from one specimen, so these dimensions must be regarded as only approximate.

*Ventral pouch.* The ventral pouch is somewhat characteristic in

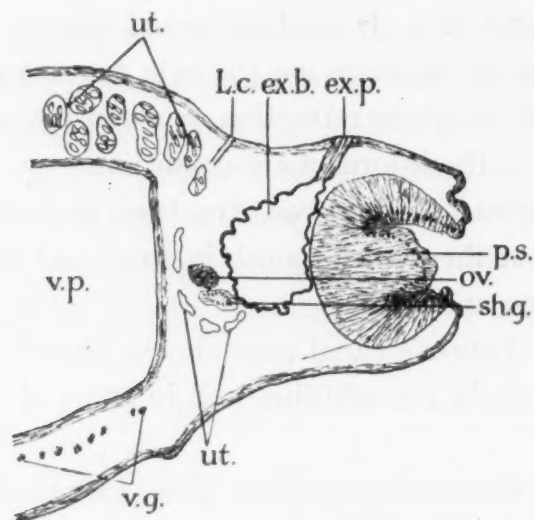


FIG. 20. *Carmyerius exoporus*, n.sp. Sagittal section of posterior end of worm. *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *L.c.*—Laurer's canal; *ov.*—ovary; *p.s.*—posterior sucker; *sh.g.*—shell gland; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch.  $\times 16$ .

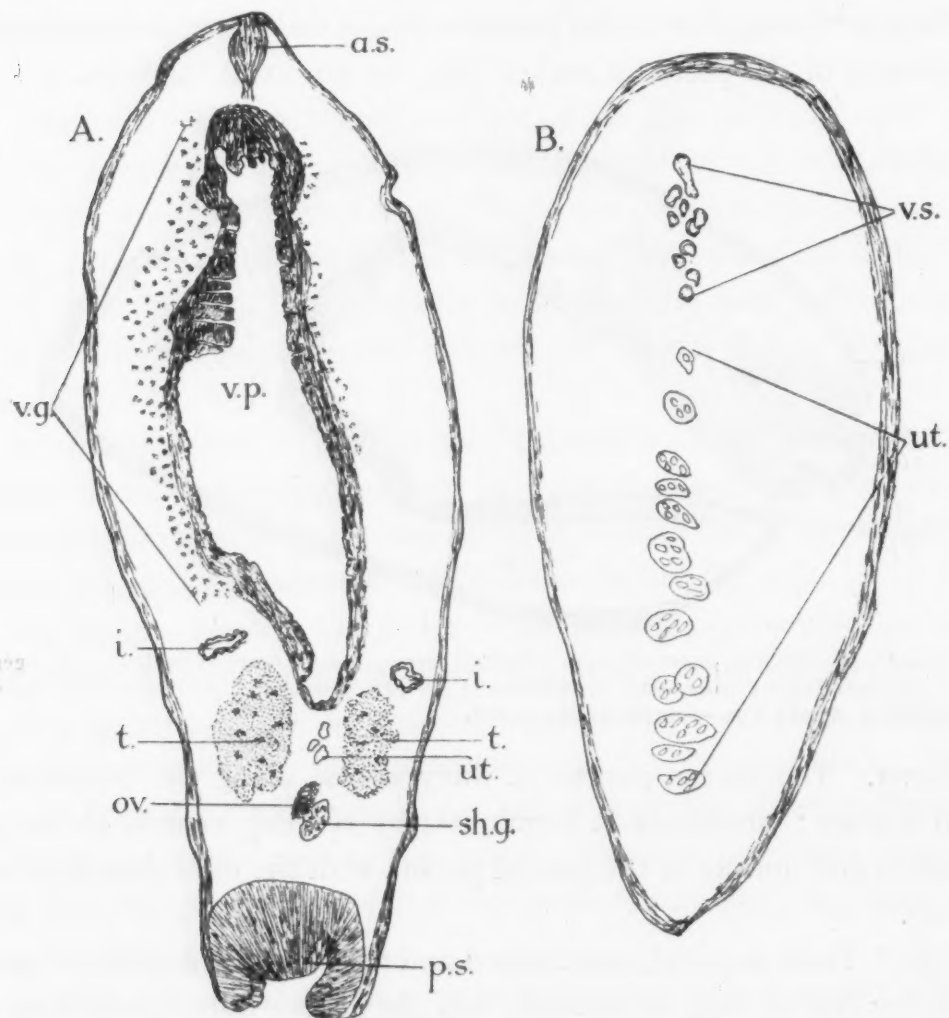


FIG. 21. *Carmyerius exoporus*. Coronal sections. *A*—About middle of worm. *B*—Near dorsal surface. *a.s.*—anterior sucker; *i.*—intestine; *ov.*—ovary; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t.*—testes; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch; *v.s.*—vesicula seminalis.  $\times 8$ .

some cases, in that on cross section about its middle it often shows six points or angles; but it is subject to so many variations that this cannot be regarded as a diagnostic character. These variations are well shown in figs. 23, 24 and 25.

#### DIAGNOSIS

As this worm is easily distinguished by the fact that the genital pore opens outside the ventral pouch, the name *Carmyerius exoporus*, n.sp., is suggested.

Genus *Wellmanius*, Stiles and Goldberger, 1910.

This genus was established by Stiles and Goldberger to include a new species *Wellmanius wellmani* which was obtained from the stomach of a reed bok (*Cervicapra bohor*) at Benguella, West Africa. The sole distinguishing character between *Wellmanius* and *Carmyerius* is that in the former the first part of the vesicula seminalis is straight, and in the latter it is coiled from the commencement. This seems a very small point on which to establish a genus, especially when it is found that the beginning of the vesicula seminalis cannot be made out with accuracy in most gravid specimens whatever the species may be, because it is overlaid by the uterus. *W. wellmani* appears to the writer to be synonymous with *C. spatiosus*.

Family *GASTRODISCIDAE*, Stiles and Goldberger, 1910.

*Definition*.—*Amphistomata*: body usually flattened and divided into anterior and posterior portions.

#### KEY TO GENERA

1. Anterior portion large and flat, and posterior portion smaller and spherical  
*Homalogaster*
- Anterior portion small and conical, posterior portion large and flat ... 2
2. Genital pore on anterior portion, ventral surface of posterior portion not covered with papillae ... *Gastrodiscoides*
- Genital pore on posterior portion, ventral surface of posterior portion covered with papillae ... *Gastrodiscus*

Genus *Gastrodiscus*, Leuckart, 1877.

*Definition*.—*Gastrodiscidae*: anterior portion small and conical, posterior portion large and flat; genital pore on posterior portion, ventral surface of posterior portion covered with papillae.

Type species *Gastrodiscus aegyptiacus* (Cobbold, 1877), Looss, 1896.

#### KEY TO SPECIES

- Genital pore less than 1 mm. from anterior border of posterior portion *G. aegyptiacus*  
 Genital pore over 1 mm. from anterior border of posterior portion *G. secundus*

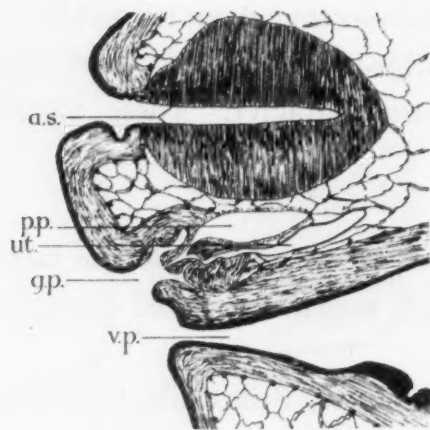


FIG. 22. *Carmyerius exoporus*, n.sp. Sagittal section on enlarged scale to show genital pore. a.s.—anterior sucker; g.p.—genital pore; p.p.—pars prostatica; ut.—uterus; v.p.—ventral pouch.  $\times 30$ .

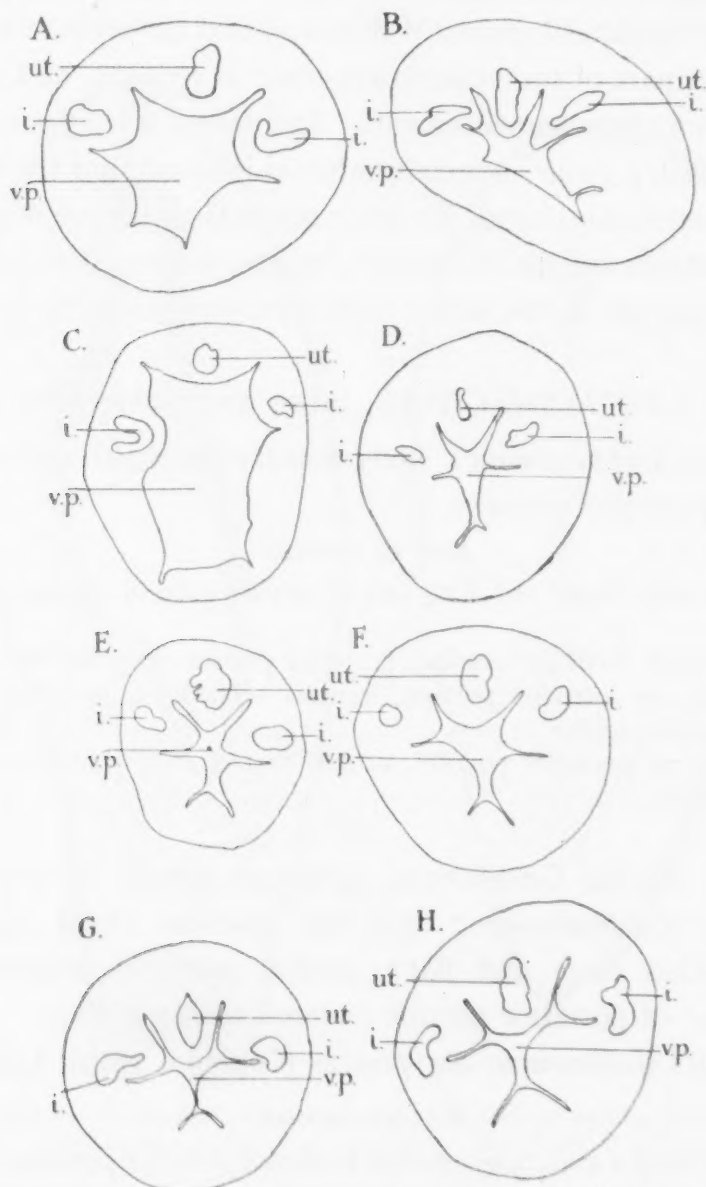


FIG. 23. *Carmyerius exoporus*, n.sp. Transverse section of eight specimens near the middle showing variation in shape of ventral pouch. i.—intestine; ut.—uterus; v.p.—ventral pouch.  $\times 9$ .



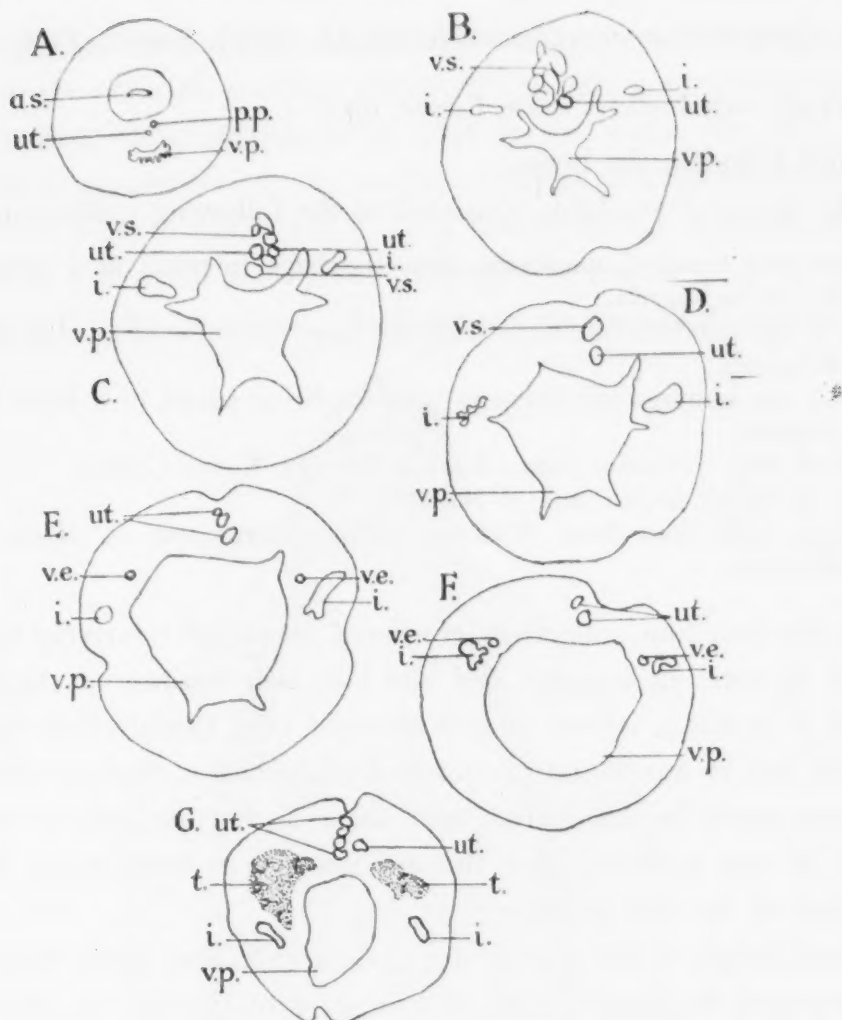


FIG. 24. *Carmyrius exoporus*, n.sp. Seven transverse sections of a single worm at different levels showing alteration in shape of ventral pouch. a.s.—anterior sucker; i.—intestine; p.p.—pars prostatica. t.—testes; ut.—uterus; v.e.—vas efferens; v.p.—ventral pouch; v.s.—vesicula seminalis.  $\times 12$ .

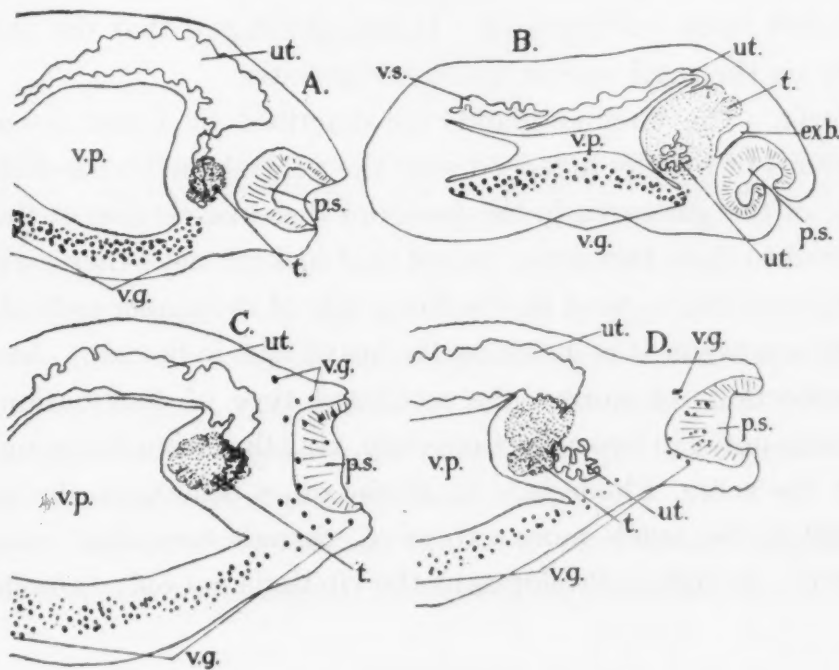


FIG. 25. *Carmyrius exoporus*, n.sp. Sagittal sections of four specimens to show differences in shape of ventral pouch. A, C, and D—Gravid worms. B—Immature worm. ex.b.—excretory bladder; p.s.—posterior sucker; t.—testis; ut.—uterus; v.g.—vitelline gland; v.p.—ventral pouch; v.s.—vesicula seminalis.  $\times 9$ .



*Gastrodiscus aegyptiacus* (Cobbold, 1877), Looss, 1896.

SYNONYMY:—*Gastrodiscus minor*, Leiper, 1913.

First found in the horse.

The material available consisted of the following collections:—

1. Over one hundred specimens from the large intestine of a pony at Ilorin, Northern Nigeria.
2. Over one hundred specimens from the large intestine of a zebra (*Equus* sp.) in Rhodesia.
3. Over one hundred specimens in poor condition passed by a horse in Northern Nigeria.
4. About fifty specimens from a horse at Nairobi, Kenya Colony.
5. Six specimens from a mule at Nairobi.
6. Three collections from wart-hogs (*Phacochoerus* sp.) in Ngoa, North-East Rhodesia.

As the first four collections consisted mainly of relatively large worms (about 15 mm. in length), and the last four mainly of smaller worms (about 9-12 mm.), it was at first thought that two distinct species were present, but it was found on detailed examination that no differences in anatomy could be discovered, and, as in both types gravid worms were found, it was assumed that the difference is nothing more than a size variation of the one species.

Looss (1896) in his description of *G. aegyptiacus* states that the testes are arranged diagonally, with the anterior of the two on the right side and the posterior on the left; the ovary was on the left side behind the posterior testis. Among specimens examined in the present instance, it was found that the relative position of the testes varied and that sometimes the left testis was anterior. It was also noted that the ovary was invariably on the same side as the anterior testis.

*Vitellaria*. The vitelline glands are described by Looss as composed of two groups of follicles arranged near the ventral surface, and along the outer side of the gut caeca in the posterior disc-like portion of the worm, and confined to these two areas, except that in some cases they may spread inwards so as nearly to meet on the dorsal side of the hinder ends of the gut caeca (this arrangement is shown by the heavy dots in fig. 26). Among the present collections of worms, the restricted type of distribution of the vitellaria was noted in two collections only, viz., that from the pony, Ilorin, and from the zebra, Rhodesia. In all the other collections the vitellaria were found to be much more extensive, though somewhat variable in distribution. In fullest development the vitellaria not only extended right

across the dorsal surface of the posterior disc-like part for its whole length, but also into the cephalic portion, where at times they were very thickly massed and completely encircled this part of the worm (fig. 26). The number of gland groups varied very much in different individuals, especially in the cephalic part of the worm. In a few cases the vitellaria in the caudal part of the worm were almost completely confined to the inter-caecal field on the dorsal aspect. In these worms the vitellaria extended

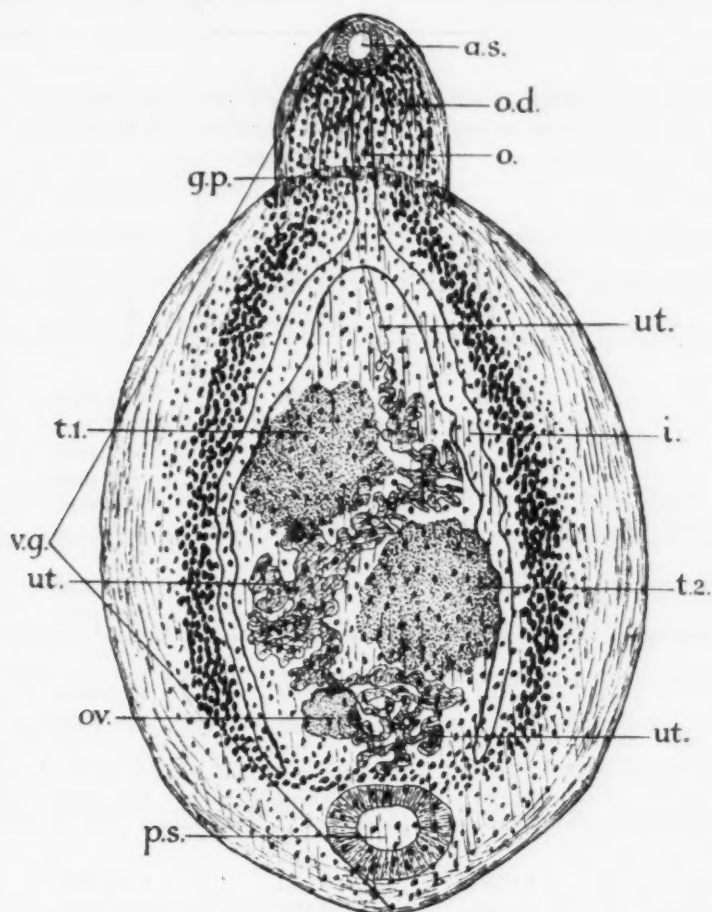


FIG. 26. *Gastrodiscus aegyptiacus*. Dorsal surface uppermost. *a.s.*—anterior sucker; *o.*—oesophagus; *o.d.*—oral diverticulum; *g.p.*—genital pore; *i.*—intestine; *ov.*—ovary; *p.s.*—posterior sucker; *t.1.*—anterior testis; *t.2.*—posterior testis; *ut.*—uterus; *v.g.*—vitelline gland.  $\times 6$ .

as a single broad band from the anterior end to the posterior end of the worm. It might be considered that these differences are of specific value, but in view of the great variation found in the distribution of the vitellaria in all other species of *Amphistomata* that the writer has examined, one is compelled to consider them of no specific value.

With the object of determining the exact position of the genital pore in relation to the anterior edge of the caudal portion of the worm, nineteen

specimens from the various collections were examined (see Table X). It was not possible to examine more specimens in this particular point, because the edge of the caudal part is usually incurved, so that it requires considerable pressure, and hence distortion, to flatten it out sufficiently to measure the distance between the genital pore and this edge.

TABLE X

Distance between genital pore and anterior edge of discal portion of worm

Host	Type of worm (large or small)	Distance of genital pore from anterior edge of caudal part	Total length of discal part	Ratio between the two foregoing dimensions
Wart-Hog ... ..	Small	572 $\mu$	9.9 mm.	1 : 17.3
		625 $\mu$	11.0 mm.	1 : 17.6
		416 $\mu$	9.1 mm.	1 : 21.8
		416 $\mu$	8.9 mm.	1 : 21.4
Wart-Hog ... ..	Small	468 $\mu$	10.0 mm.	1 : 21.4
		781 $\mu$	11.4 mm.	1 : 14.6
		572 $\mu$	10.4 mm.	1 : 18.2
		625 $\mu$	9.8 mm.	1 : 15.6
		400 $\mu$	8.3 mm.	1 : 20.75
Mule, Nairobi ... ..	Small	468 $\mu$	8.8 mm.	1 : 18.9
		572 $\mu$	9.6 mm.	1 : 16.8
Zebra ... ..	Large	781 $\mu$	13.3 mm.	1 : 17.1
		729 $\mu$	13.0 mm.	1 : 17.8
Pony, Ilorin ... ..	Large	760 $\mu$	13.5 mm.	1 : 17.8
Horse, Nairobi ... ..	Large	625 $\mu$	11.3 mm.	1 : 18.1
		833 $\mu$	12.5 mm.	1 : 15.0
		677 $\mu$	12.0 mm.	1 : 17.7
		639 $\mu$	11.25 mm.	1 : 17.8
		625 $\mu$	13.75 mm.	1 : 22.0

It will be noted from the above table that the distance between the genital pore and the anterior edge of the caudal part of the worm varies considerably. Another point which this series of measurements brought out is that although on the whole the two types are fairly distinct, relatively small worms may be found in bottles in which the predominating number are large, and in bottles that contain nearly all small worms a few relatively large ones occur, and these exceptional specimens tend to unite the two types into a complete whole. From these observations it is

concluded that the species *G. aegyptiacus* is subject to considerable size variation, and that the distance of the genital pore, although further from the edge of the caudal part of the worm in large examples than it is in small ones, is variable.

*Gastrodiscus minor*, Leiper, 1913.

This species is recorded by Leiper (1913) as new, the host being the pig in Uganda. All Leiper gives in the way of description is the following passage :

'This small fluke resembles closely the African *G. aegyptiacus* (*vel sonsinot*), which is so frequently met with in horses in Egypt and in West Africa : it differs, however, in a number of respects, particularly in the nearness of the genital pore to the edge of the ventral disc-like expansion.'

From this it is rather difficult to deduce in what the differences between *G. minor* and *G. aegyptiacus* consist, especially if reference is made to Table X. *G. minor* is therefore regarded as a synonym of *G. aegyptiacus*.

*Gastrodiscus secundus*, Looss, 1907.

The material available for study consisted of a single collection of about twenty specimens. It was from the same collection that Looss obtained the material he used in his description of the species.

Examination of this material did not reveal any differences from the original description by Looss (1907), except that the position of the testes and ovary varied in the same manner as has been described in *G. aegyptiacus*. The worm can at once be distinguished from *G. aegyptiacus* by the position of the genital pore. Fig. 27 shows the essential anatomical details.

Genus *Gastrodiscoides*, Leiper, 1913.

*Definition.*—*Gastrodiscidae* : anterior portion small and conical, genital pore on anterior portion, no papillae on ventral surface of posterior portion.

Type species *Gastrodiscoides hominis* (Lewis and McConnal, 1876), Leiper, 1913.

Only one species described.

SYNONYMY :—

*Amphistoma hominis*, Lewis and McConnal, 1876.

*Gastrodiscus hominis*, Ward, 1903.

First found in the colon of man in Assam.



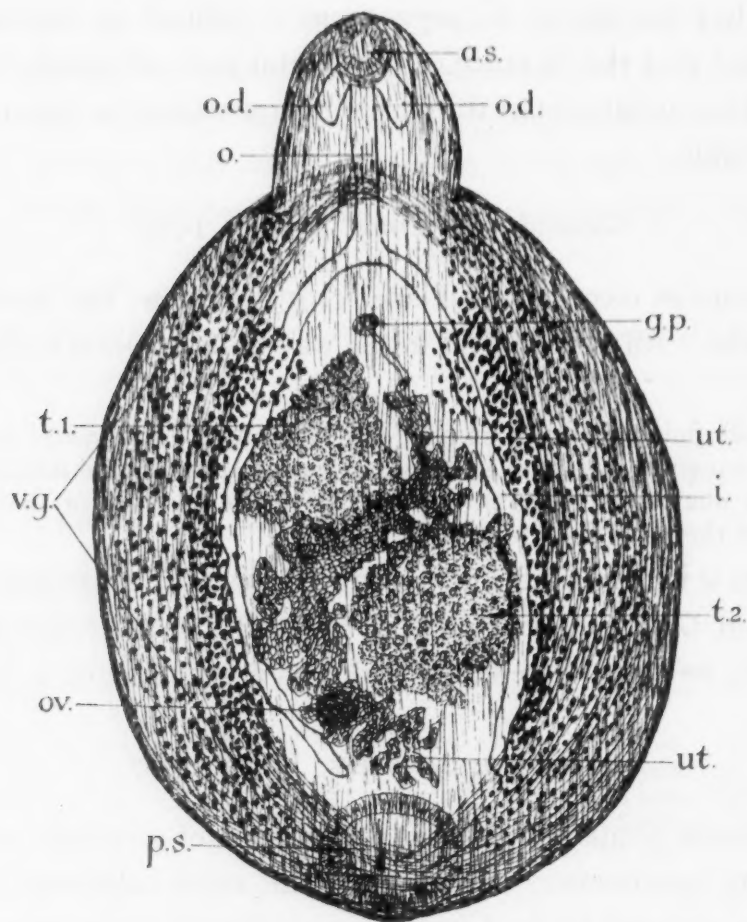


FIG. 27. *Gastrodiscus secundus*. Dorsal surface uppermost. *a.s.*—anterior sucker; *g.p.*—genital pore; *i.*—intestine; *o.*—oesophagus; *o.d.*—oral diverticulum; *ov.*—ovary; *p.s.*—posterior sucker; *t.1.*—anterior testis; *t.2.*—posterior testis; *ut.*—uterus; *v.g.*—vitelline gland.  $\times 12$ .

*Gastrodiscoides hominis* (Lewis and McConnal, 1876), Leiper, 1913.

The material available for study consisted of:—

Two collections from Annam consisting of nine whole worms and six specimens cut and mounted in serial section. This is the same material as Stephens (1906) used in his description of the worm.

Leiper describes and figures (fig. 35) a prominent genital papilla with the male and female ducts opening separately near its tip. Figs. 28 and 29 in the present paper were drawn from two sectioned specimens cut by Stephens. In fig. 28, it will be noted that there is a deep atrium with no sign of a papilla, the openings of the uterus and vas deferens being widely separated from one another and lying at the deepest part of the atrium. This represents a worm with the papilla completely retracted. In fig. 29 there is a bulbous papilla partly protruded, with a small atrium surrounding



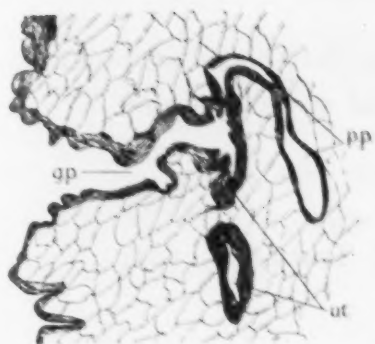


FIG. 28. *Gastrodiscoides hominis*. Sagittal section of genital pore, with papilla fully retracted. g.p.—genital pore; p.p.—pars prostatica; ut.—uterus  $\times 30$ .

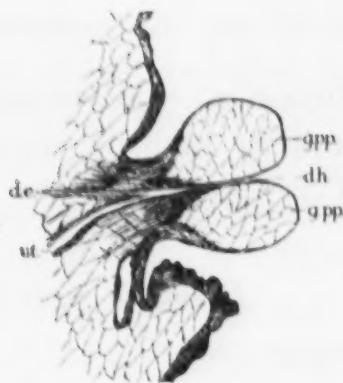


FIG. 29. *Gastrodiscoides hominis*. Sagittal section of genital pore, with papilla partly extruded. d.e.—ductus ejaculatorius; d.h.—ductus hermaphroditicus; g.p.p.—genital papilla; ut.—uterus.  $\times 30$ .

its base, the male and female ducts uniting within the papilla and opening at its tip by a common duct, in the usual way. These two drawings taken in conjunction with Leiper's figure indicate that the presence or absence of a prominent genital papilla, or of a genital atrium, are purely matters of chance, and are of no more diagnostic value in this instance than in any other species of the group *Amphistomata*.

#### Genus *Homalogaster*, Poirier, 1883.

*Definition*.—*Gastrodiscidae*: anterior portion large and flat, posterior portion smaller and spherical.

Type species *Homalogaster poloniae*, Poirier, 1883.

Only one species described.

#### *Homalogaster poloniae*, Poirier, 1883.

##### SYNONYMY:—

*Homalogaster poirieri*, Giard and Billet, 1892.

*Homalogaster philippinensis*, Stiles and Goldberger, 1910.

First found in stomach of *Palonia frontalis*, in Java.

The writer has not had the opportunity of examining any material of this species, but Railliet, Henry, and Bauche (1914) discuss its synonymy, and it is from their paper that it has been taken.

*Brumptia*, Travassos, 1921

*Definition.*—*Amphistomata* : with paired caudal appendages containing most of the vitellaria, cirrus pouch and genital sucker present.

Type species *Brumptia gigas*.

*Brumptia gigas* (MacCallum, 1917) Travassos, 1921

SYNONYMY :—

*Cladorchis gigas*, MacCallum, 1917.

This worm was found on two occasions in the stomach of a rhinoceros at Ngoa, North-east Rhodesia.\* Each collection consists of about twenty-five specimens.

## EXTERNAL ANATOMY

*Size and shape.* The worms were of slightly different size in the two collections, those in one bottle being all about 15 mm. in length by 9 mm. in breadth, and those in the other bottle about 12 mm. in length by 7 mm. in breadth. Gravid worms were found in both collections, but as detailed examination revealed no differences other than size, it is considered that there was only one species. The worms consist of two portions distinctly separated, an anterior conical portion and a posterior part consisting of two crescentic flaps. The anterior part is conical in shape with a definite ventral curve; the ventral surface is almost flat from side to side, whilst the dorsal surface is domed both laterally and antero-posteriorly.

The posterior sucker is slightly in front of the posterior extremity of the body of the worm, and is situated entirely on the ventral surface, and directed ventrally. About midway between the two suckers in the mid-line of the ventral surface is the genital pore. Comparison of figs. 5 and 6, Plate VIII, will show how the appearance of the genital pore varies with retraction or extrusion of the genital papilla.

The most characteristic feature of the worm is the presence of two large

\* The above description was written before I became aware that MacCallum (1917) had described a worm which is apparently the same species. MacCallum obtained his material from the African elephant (*Loxodon africanus*), he named it *Cladorchis gigas*. The Liverpool material seems to be identical with MacCallum's in all anatomical details, but the two collections are a little different in size, MacCallum's worms are 21 mm. in length, and ours are from 12 mm. to 15 mm. in length. In view of the results obtained in other species this slight difference is not considered to be of importance. Travassos (1921) created a new genus *Brumptia* to accommodate MacCallum's species *C. gigas*, leaving it in the sub-family *Cladorchini*, but the two caudal flaps containing the vitelline glands are so strikingly different from any other genus in this sub-family that I consider it preferable to leave it as a genus of uncertain position.

crescentic caudal appendages. These arise from the postero-lateral borders, extend laterally as far forward as the anterior border of the ventral sucker, and are separated from one another behind the sucker by a deep notch. In full extension they measure about 5 mm. in length, but as a rule, their borders are incurved towards the ventral surface, so that they appear somewhat shorter and tend to overlap the posterior sucker.

#### INTERNAL ANATOMY

On account of the thickness of these worms, very little could be ascertained in whole specimens cleared in carbolic acid, so that the following description is mainly based on a study of serial sections cut in sagittal, coronal, and transverse planes.

*Muscular system.* The muscular system, as a whole, is very similar to other species, but in certain special structures it departs from the usual type, and these differences will be dealt with under the appropriate organs.

*Nervous system.* This system was not investigated.

*Excretory system.* The excretory bladder is large when in a state of distension and occupies the whole of the posterior part of the worm between the posterior sucker and the dorsal surface. The excretory canal in the specimens examined ran almost directly posteriorly to open in the mid-line near the posterior end of the dorsal surface (fig. 30, B).

*Anterior sucker.* The anterior sucker is a thick walled muscular structure surrounding the oral cavity; about the junction of the middle and posterior thirds there is an annular constriction at which point two large muscular diverticula arise and run in a dorsal and slightly posterior direction (figs. 31, A and 32, C).

*Oesophagus and intestines.* The oesophagus is of the usual type, its muscular wall becoming slightly thicker as the posterior end is approached. In the specimens examined, it curved at first ventrally, and then turning abruptly towards the dorsal surface, divided into the gut caeca in front of the cirrus pouch (fig. 30, B). The caeca pursue a wavy course along each side of the worm and end in the dorsal part of the caudal flap (fig. 30, A).

*Genitalia. Testes.* These are large oval organs lying side by side in the lateral fields somewhat nearer to the ventral than to the dorsal surface. They lie in front of the posterior sucker, and the posterior part of the cirrus pouch is between their anterior ends (figs. 30, A, 31, B, and 32, B). No external lobing is visible, but in sections each testis is seen to be divided

up into numerous separate acini, the whole being surrounded by a loose connective tissue capsule. Each testis is about 3.5 mm. in diameter.

*Vasa efferentia.* These arise from the antero-mesial aspect of each testis, and suddenly dilate into broad thin-walled tubes, which, running upwards and inwards over the posterior wall of the cirrus pouch, enter this structure on its dorso-posterior aspect by two narrow tubes lying close to each other. When they reach the inner aspect of the wall of the cirrus pouch they unite to form the vas deferens (figs. 30, 31, C and 32, A).

*Vas deferens.* The vesicula seminalis is a dilated, thin-walled sac

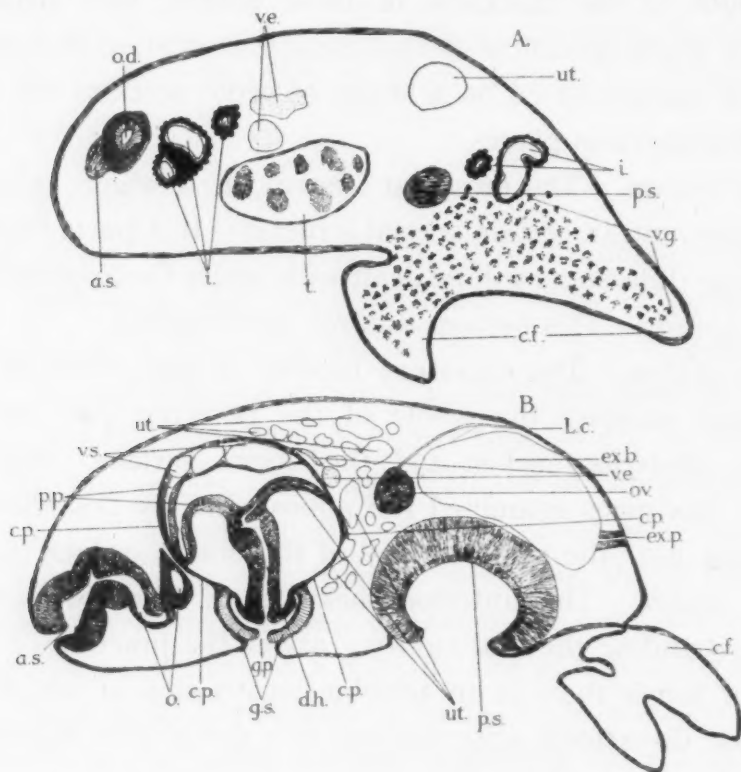


FIG. 30. *Brumptia gigas*. Sagittal sections. A—To one side of mid-line. B—In mid-line. a.s.—anterior sucker; c.f.—caudal flap; c.p.—cirrus pouch; d.b.—ductus hermaphroditicus; ex.b.—excretory bladder; ex.p.—excretory pore; g.p.—genital pore; g.s.—genital sucker; L.c.—Laurer's canal; o.—oesophagus; o.d.—oral diverticulum; ov.—ovary; p.p.—pars prostatica; p.s.—posterior sucker; t.—testis; ut.—uterus; v.e.—vas efferens; v.s.—vesicula seminalis.  $\times 6$ .

which runs along the dorsal wall of the cirrus pouch, being held in place by some strands of connective tissue (figs. 30, B and 31, B). Near the anterior end of the cirrus pouch on its dorsal surface, the vesicula seminalis passes into the pars prostatica. The pars prostatica runs ventrally for some distance close along the anterior wall of the cirrus pouch, and then leaving the wall of the cirrus pouch turns sharply towards the dorsal surface, curving posteriorly until it ends by uniting with the uterus near the centre of the pouch (fig. 30, B). The pars prostatica is thickly



surrounded by cells for its whole course and no pars muscosa could be distinguished. The genital papilla appeared as a long muscular tube running from about the centre of the cirrus pouch towards the ventral surface (figs. 30, B and 31, B), but in all the specimens sectioned it was in a state of retraction, and would probably appear quite different in sections of a worm like that shown in Plate VIII, fig. 5, where it is obviously protruded.

*Cirrus pouch.* The cirrus pouch is a spherical organ about 4 mm. in diameter. It lies near the centre of the worm slightly towards its anterior end. Its wall is composed of loosely laminated muscular fibres. That part of the cirrus pouch which is not occupied by sex ducts is filled with loose areolar tissue.

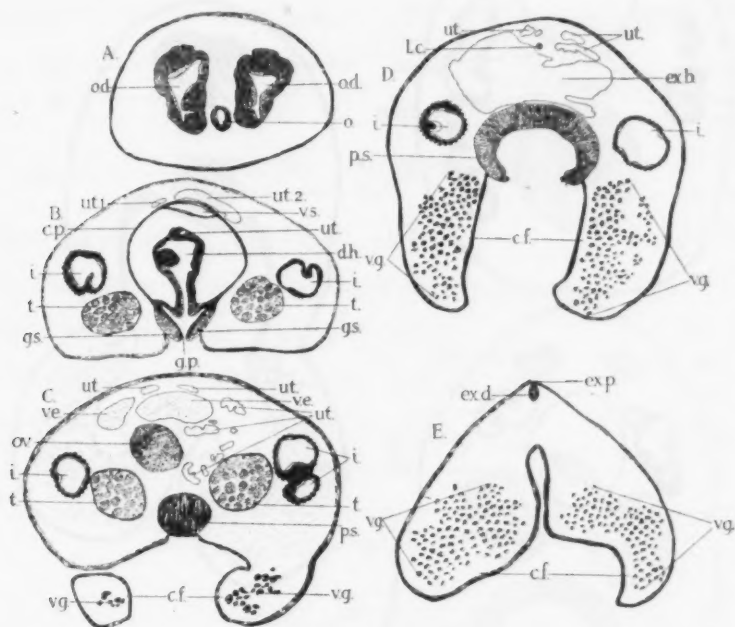


FIG. 31. *Brumptia gigas*. Transverse sections. A—Near anterior end. B—Through cirrus pouch. C—Through ovary. D—Through middle of posterior sucker. E—Through excretory pore. c.f.—caudal flap; c.p.—cirrus pouch; d.b.—ductus hermaphroditicus; ex.b.—excretory bladder; ex.d.—excretory duct; ex.p.—excretory pore; g.p.—genital pore; g.s.—genital sucker; i.—intestine; L.c.—Laurer's canal; o.—oesophagus; o.d.—oral diverticulum; p.s.—posterior sucker; t.—testis; ut.—uterus; ut1.—ascending branch of uterus; ut2.—descending branch of uterus; v.e.—vas efferens; v.g.—vitelline gland; v.s.—vesicula seminalis.  $\times 44$ .

*Genital pore.* This is provided with a definite small sucker surrounding its opening. This sucker is much more definitely marked off from the subcuticular muscle than in the case of the genus *Cotylophoron*, as it is composed of radially arranged fibres quite distinct from the subcuticular muscle; this is shown in figs. 31, B and 30, B, in both of which the genital papilla is seen lying within the genital sucker and surrounded by a small atrium, which would obviously disappear if the papilla were extruded.



**Ovary.** This lies towards the dorsal surface between the testes and slightly to one side of the mid-line. It is a circular organ with no special characters (figs. 30, B, 31, C, and 32, A). The shell gland lies on the mesial aspect of the ovary (fig. 32, A).

**Laurer's canal.** Laurer's canal runs dorsally from the shell gland and, curving posteriorly over the anterior end of the excretory bladder, it opens in the mid-line above the middle of the bladder and far in front of the excretory pore (fig. 30, B).

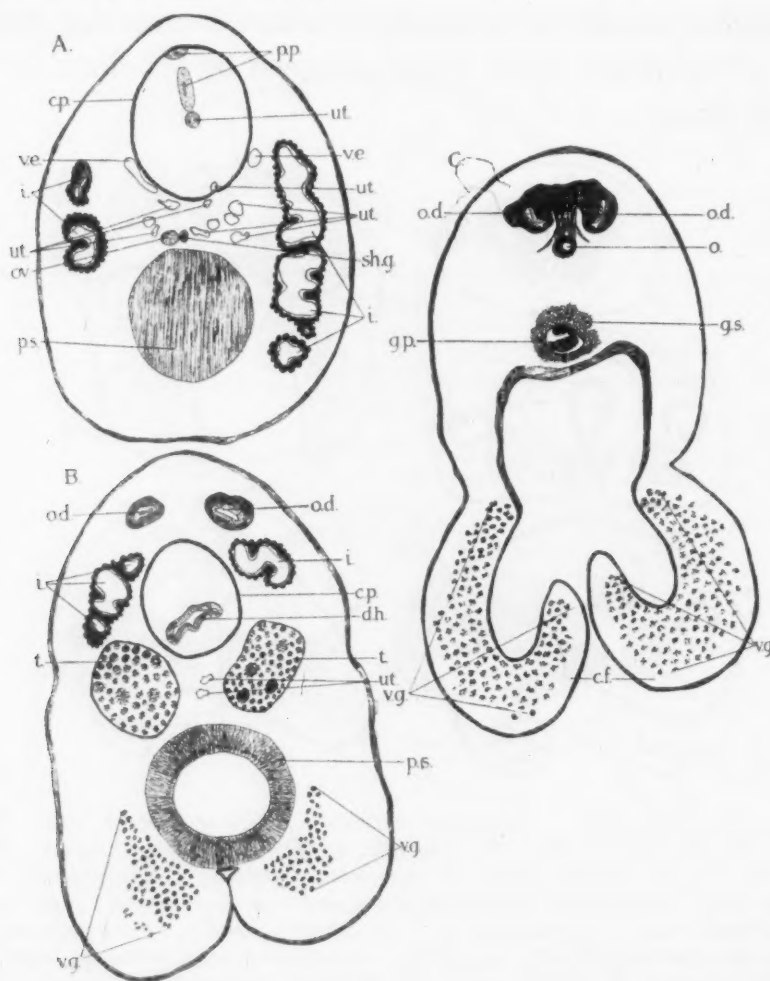


FIG. 32. *Brumptia gigas*. Coronal sections. A—Towards dorsal surface. B—About the middle. C—Near the ventral surface. c.p.—cirrus pouch; d.b.—ductus hermaphroditicus; g.p.—genital pore; g.s.—genital sucker; i.—intestine; o.—oesophagus; o.d.—oral diverticulum; ov.—ovary; p.p.—pars prostatica; p.s.—posterior sucker; sh.g.—shell gland; t.—testis; ut.—uterus; v.e.—vas efferens; v.g.—vitelline gland.  $\times 4\frac{1}{2}$ .

**Vitellaria.** The vitelline glands consist of numerous collections of follicles which lie nearly entirely within the two caudal appendages (figs. 30, A, 31, C, D and E, 32, B and C). A few groups of follicles were seen near the ventral surface of the worm in front of the posterior sucker.

*Uterus.* For the first part of its course the uterus shows no special differences from the usual type. But after it reaches the anterior border of the cirrus pouch on its dorsal aspect, it turns posteriorly and runs back, still close to the dorsal wall of the cirrus pouch, and following the curve of the posterior wall of this organ comes close to the ventral surface; it then turns sharply dorsally and enters the posterior wall of the cirrus pouch about its middle. From this point, it runs anteriorly through the pouch to unite near the centre with the end of the pars prostatica (fig. 30, B).

*Eggs.* The eggs removed from the uterus of one specimen were oval and operculated and measured  $112\mu$  to  $116\mu$  in length by  $76\mu$  to  $70\mu$  in breadth, but it must be remembered that measurements of eggs taken from the uterus of fixed worms are only very approximate.

#### SPECIES INQUIRENDAE

*Amphistomum papillatum*, Cobbold, 1882.

Found in intestine of *Elephas indicus*, India.

*Amphistomum tuberculatum*, Cobbold, 1875.

Found in intestine of *Bos taurus*, India.

*Amphistomum emarginatum*, Diesing, 1839.

Found in intestine of *Nictipithecus trivirgatus*, Brazil.

#### CONCLUSION

As a result of an exhaustive examination of a very large collection of material comprising in many instances some hundreds of specimens, and of a careful and critical study of the monographs of Fiscoeder and of Stiles and Goldberger, the conclusion is reached that many of the species described by the former, and all except one of those described by the latter authors, are merely synonyms of earlier species. It appears to the writer that the authors have fallen in error owing to the fact that they confined themselves to the examination of limited material, in some cases to the examination of a single non-gravid worm, or even in one or two instances to that of a series of sections of a single specimen. It is only when a long series of specimens is examined that one realises to what extent individual variations occur.

## LIST OF AMPHISTOMES ARRANGED UNDER THEIR HOSTS

HOST	PARASITE	LOCATION
<i>Manatus exunguis</i> ...	<i>Chiorchis fabaceus</i> ...	Intestine.
" <i>latirostris</i> ...	" " ...	"
<i>Tapirus americanus</i> ...	<i>Cladorchis asper</i> ...	"
	<i>Cladorchis pyriformis</i> ...	"
<i>Equus caballus</i> ..	<i>Gastrodiscus aegyptiacus</i> ...	"
	<i>Pseudodiscus collinsi</i> ...	"
	<i>Gastrodiscus secundus</i> ...	"
" <i>zebra</i> ...	" <i>aegyptiacus</i> ...	"
" <i>mulus</i> ...	" " ...	"
<i>Rhinoceros</i> sp. (Rhodesia) ...	<i>Brumptia gigas</i> ...	?
<i>Phacochoerus</i> sp. ...	<i>Gastrodiscus aegyptiacus</i> ...	"
(North-East Rhodesia)		
<i>Sus</i> sp. (Annam.) ...	<i>Gastrodiscoides hominis</i> ...	"
<i>Dicotyles albirostris</i> ...	<i>Cladorchis giganteus</i> ...	"
" <i>labiatus</i> ...	" " ...	"
" <i>torquatus</i> ...	" " ...	"
	<i>Taxorchis schistocotyle</i> ...	"
<i>Hippopotamus amphibius</i> ...	<i>Paramphistomum gigantocotyle</i> ...	Stomach.
	" <i>wagandi</i> ...	"
	" <i>buxifrons</i> ...	"
	<i>Cotylophoron cotylophorum</i> ? ...	"
	" <i>minutum</i> ...	"
	<i>Carmyerius cruciformis</i> ...	"
	<i>Paramphistomum pisum</i> ...	Intestine.
<i>Bos taurus</i> ...	<i>Paramphistomum cervi</i> ...	Stomach.
	" <i>explanatum</i> ...	"
	" <i>orthocoelium</i> ...	"
	<i>Cotylophoron cotylophorum</i> ...	"
	<i>Stephanopharynx compactus</i> ...	"
	<i>Gastrothylax crumenifer</i> ...	"
	<i>Fischoederius cobboldi</i> ...	"
	" <i>elongatus</i> ...	"
	<i>Carmyerius gregarius</i> ...	"
	" <i>spatiosus</i> ...	"
	<i>Homalogaster poloniae</i> ...	Large intestine
	<i>Amphistomum tuberculatum</i> ? ...	Intestine.
<i>Bos taurus indicus</i> ...	<i>Paramphistomum cervi</i> ...	Stomach.
	" <i>orthocoelium</i> ...	"
	<i>Cotylophoron cotylophorum</i> ...	"
	<i>Gastrothylax crumenifer</i> ...	"
	<i>Fischoederius cobboldi</i> ...	"
	" <i>elongatus</i> ..	"
	<i>Carmyerius spatiosus</i> ...	"
	<i>Paramphistomum explanatum</i> ...	Bile ducts.
<i>Bos urus</i> ...	" <i>cervi</i> ...	Stomach.
<i>Bos</i> sp. (Pagan dwarf bull), Ilorin	<i>Cotylophoron cotylophorum</i> ...	"
<i>Bos bubalus</i> ( <i>Bison europaeus</i> )	<i>Paramphistomum cervi</i> ...	"
	<i>Carmyerius gregarius</i> ...	"

<i>Bos (bubalus) caffer</i> , Africa	...	<i>Cotylophoron cotylophorum</i>	...	Stomach.
		<i>Carmyerius gregarius</i>	...	"
<i>Bos (bubalus) bubalis</i> , Asia	...	<i>Paramphistomum cervi</i>	...	"
		<i>Carmyerius gregarius</i>	...	"
<i>Palonia frontalis</i> ...	...	<i>Fischoederius cobboldi</i>	...	"
		" <i>elongatus</i>	...	"
		<i>Homalogaster paloniae</i>	...	Caecum.
<i>Anoa depressicornis</i> ...	...	<i>Fischoederius elongatus</i>	...	Stomach.
<i>Capra hircus</i> ...	...	<i>Paramphistomum cervi</i>	...	"
" sp. (India)	...	<i>Gastrothylax</i> sp. ? (immature)	...	"
" sp. (Northern Territory, Gold Coast)	...	<i>Paramphistomum</i> sp. ? (immature)	...	"
<i>Ovis aries</i> ...	...	<i>Paramphistomum cervi</i>	...	"
" sp. (Port Said)	...	" "	...	"
		<i>Cotylophoron cotylophorum</i>	...	"
		<i>Gastrothylax</i> sp. ? (immature)	...	"
" sp. (South Africa)	...	<i>Paramphistomum</i> sp. ? (immature)	...	"
" sp. (Hong Kong)	...	<i>Paramphistomum orthocoelium</i>	...	"
		<i>Gastrothylax crumenifer</i>	...	"
<i>Antilope dorcas</i> ...	...	<i>Paramphistomum cervi</i>	...	"
<i>Antilope</i> sp. (Kamerun)	...	<i>Carmyerius spatiosus</i>	...	"
<i>Cobus</i> sp. (North-east Rhodesia)	...	<i>Stephanopharynx compactus</i>	...	"
		<i>Cotylophoron cotylophorum</i>	...	"
<i>Cobus</i> sp. (Zeref)	...	<i>Cotylophoron cotylophorum</i>	...	"
<i>Cobus maria</i> ...	...	<i>Carmyerius wenyoni</i>	...	"
<i>Tragelaphus scriptus</i> ...	...	<i>Carmyerius spatiosus</i>	...	Stomach ?
" <i>spekei</i> ...	...	" <i>exoporus</i>	...	Stomach.
<i>Hippotragus equinus</i> ...	...	<i>Paramphistomum cervi</i>	...	"
" "	...	<i>Carmyerius spatiosus</i>	...	"
<i>Aepyceros melampus</i> ...	...	<i>Cotylophoron cotylophorum</i>	...	"
<i>Bubalus</i> sp. (Nyasaland)	...	" "	...	"
		<i>Paramphistomum explanatum</i>	...	"
<i>Bubalis</i> sp. (Rhodesia)	...	<i>Carmyerius spatiosus</i>	...	"
<i>Cervicapra</i> sp. (Rhodesia)	...	" "	...	"
<i>Portax tragocamelus</i> ...	...	<i>Paramphistomum cervi</i>	...	"
<i>Cervus alces</i> ...	...	" <i>cervi</i>	...	"
" <i>campestris</i> ...	...	" <i>liorchis</i>	...	"
" <i>capreolus</i> ...	...	" <i>cervi</i>	...	"
" <i>dama</i> ...	...	" "	...	"
" <i>dichotomus</i> ...	...	" <i>liorchis</i>	...	"
		<i>Balanorchis anastrophus</i>	...	"
		<i>Amphistomum lunatum</i> ?	...	Intestine.
" <i>elaphus</i> ...	...	<i>Paramphistomum cervi</i>	...	Stomach.
" <i>mexicanus</i> ...	...	" <i>liorchis</i>	...	"
" <i>namby</i> ...	...	" "	...	"
" <i>rufus</i> ...	...	" "	...	"
" <i>simplicicornis</i>	...	" "	...	"
<i>Elephas indicus</i> ...	...	<i>Pseudodiscus hawkesii</i>	...	Intestine.
<i>Loxodon africanus</i> ...	...	<i>Brumptia gigas</i>	...	"
		<i>Amphistomum papillatum</i> ?	...	"
<i>Castor fiber</i> ...	...	<i>Cladorchis subtriquetrus</i>	...	Small and large Intestine.



<i>Callithrix noctivaga</i>	...	...	<i>Amphistomum emarginatum</i> ?	...	Intestine.
<i>Cercopithecus callitrichus</i>	...	...	<i>Pseudodiscus watsoni</i>	...	Colon.
<i>Macacus cynomolgus</i>	...	...	<i>Pseudodiscus watsoni</i> ?	...	Colon.
<i>Homo sapiens</i>	...	...	<i>Gastrodiscoides hominis</i>	...	Intestine.
			<i>Pseudodiscus watsoni</i>		

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\* This paper was not consulted.



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## EXPLANATION OF PLATE V

*Paramphistomum cervi*. Photographs showing variations in size and shape exhibited by 24 specimens.  $\times 2\frac{1}{2}$ .

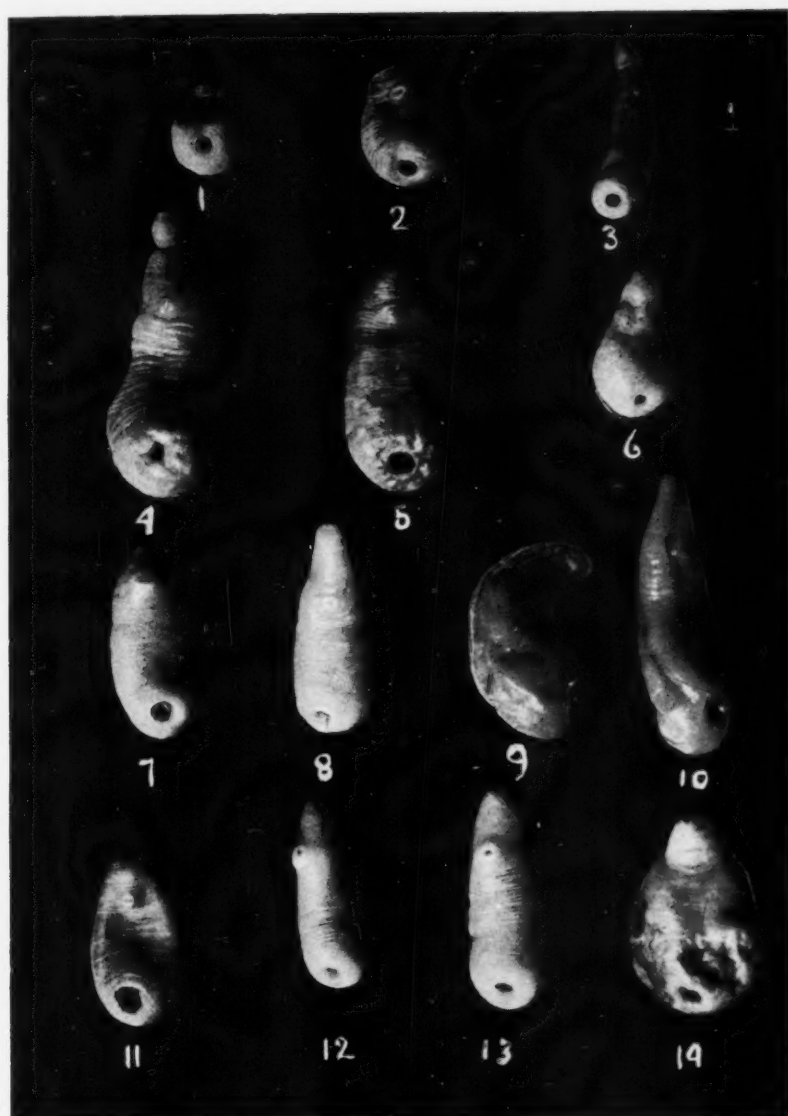


FIG. A

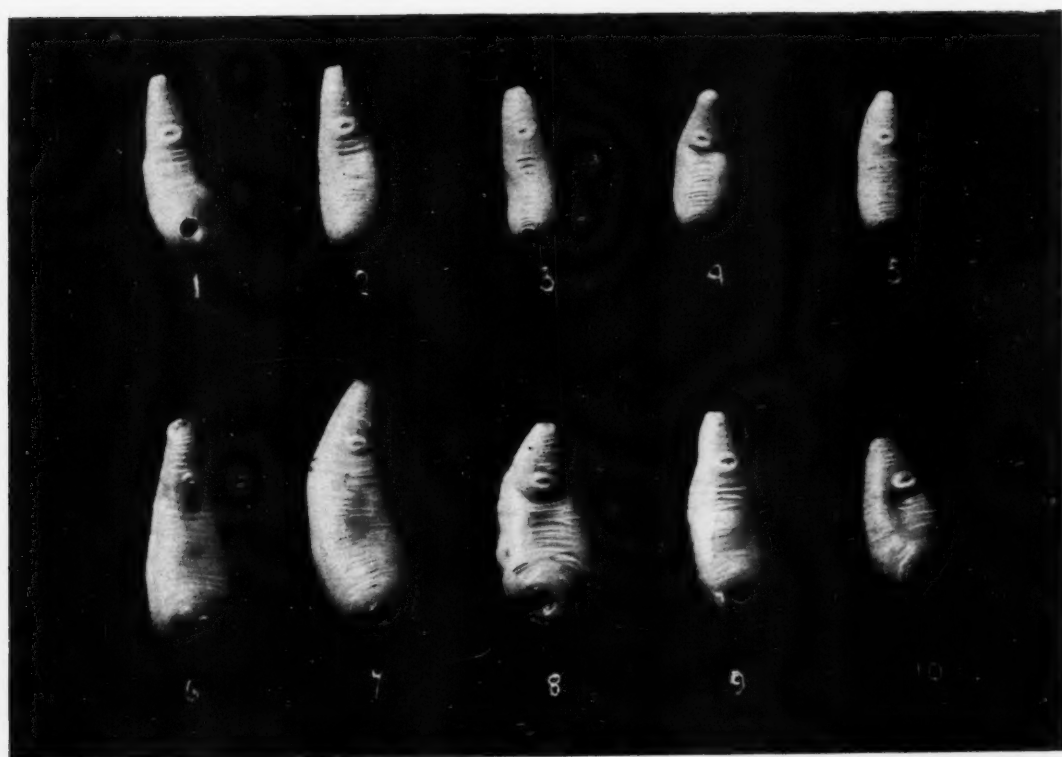


FIG. B

## EXPLANATION OF PLATE V

*Paramphistomum cervi*. Photographs showing variations in size and shape exhibited by 24 specimens.  $\times 2\frac{1}{2}$ .

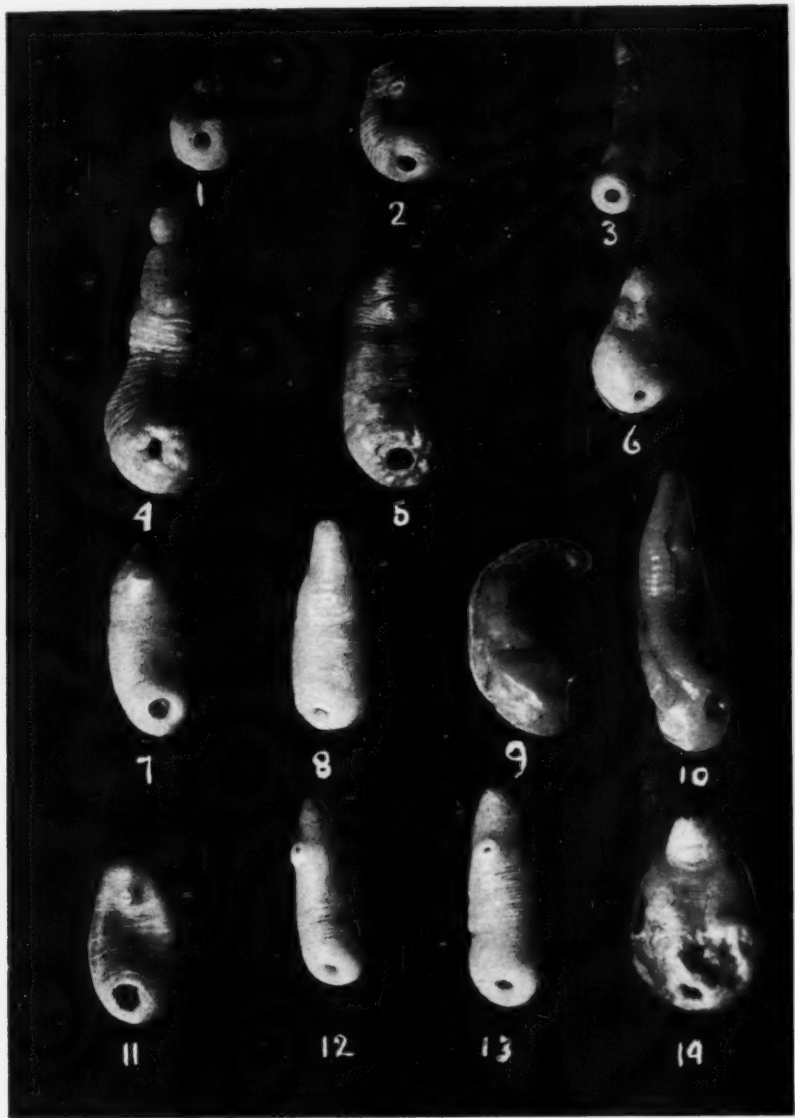


FIG. A

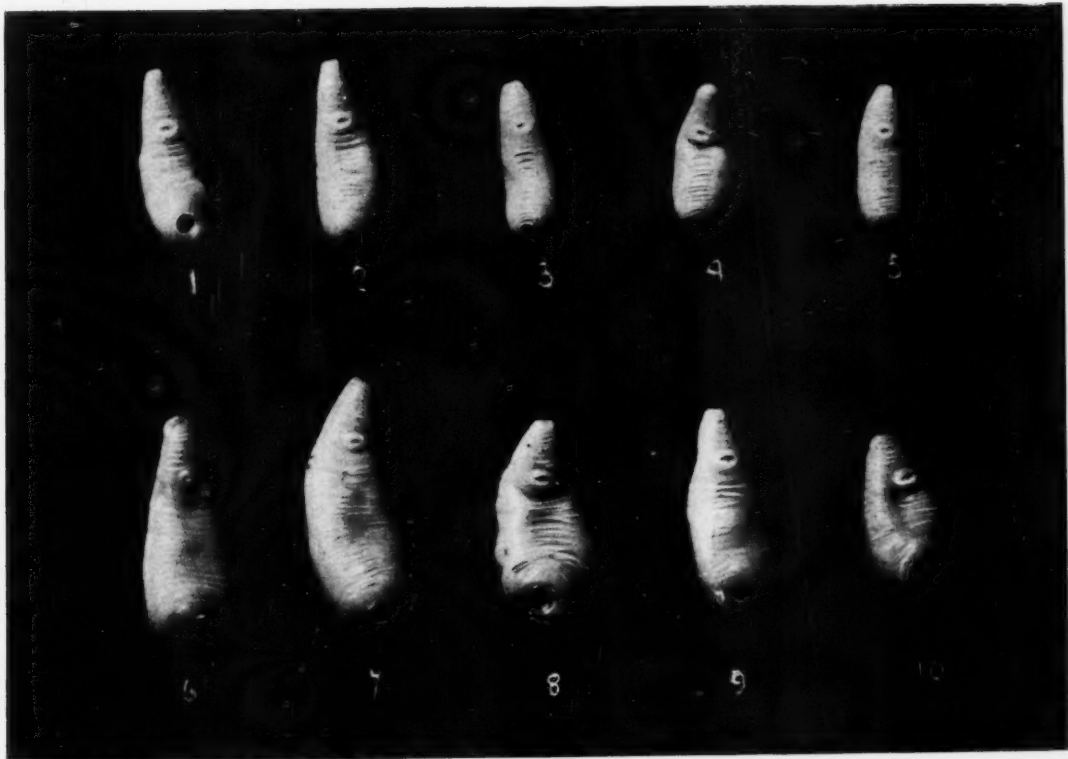


FIG. B



## EXPLANATION OF PLATE VI

Fig. A. *Paramphistomum explanatum*.  $\times 2\frac{1}{2}$ .

Fig. B. *Cotylophoron cotylophorum*.  $\times 2\frac{1}{2}$ .

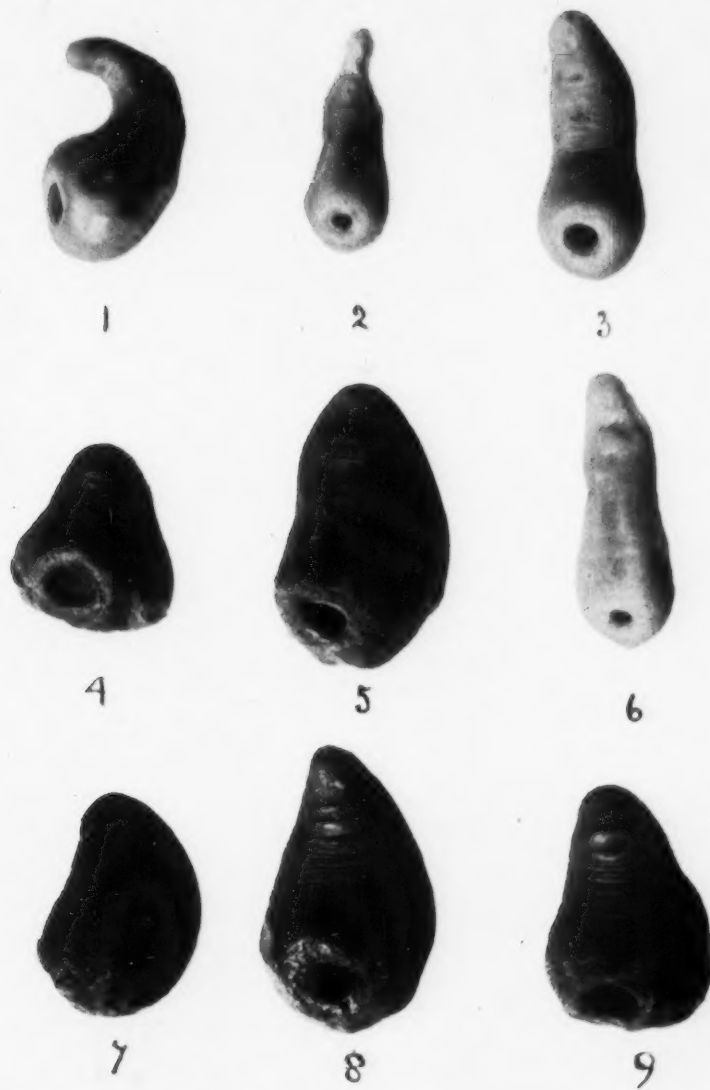


FIG. A

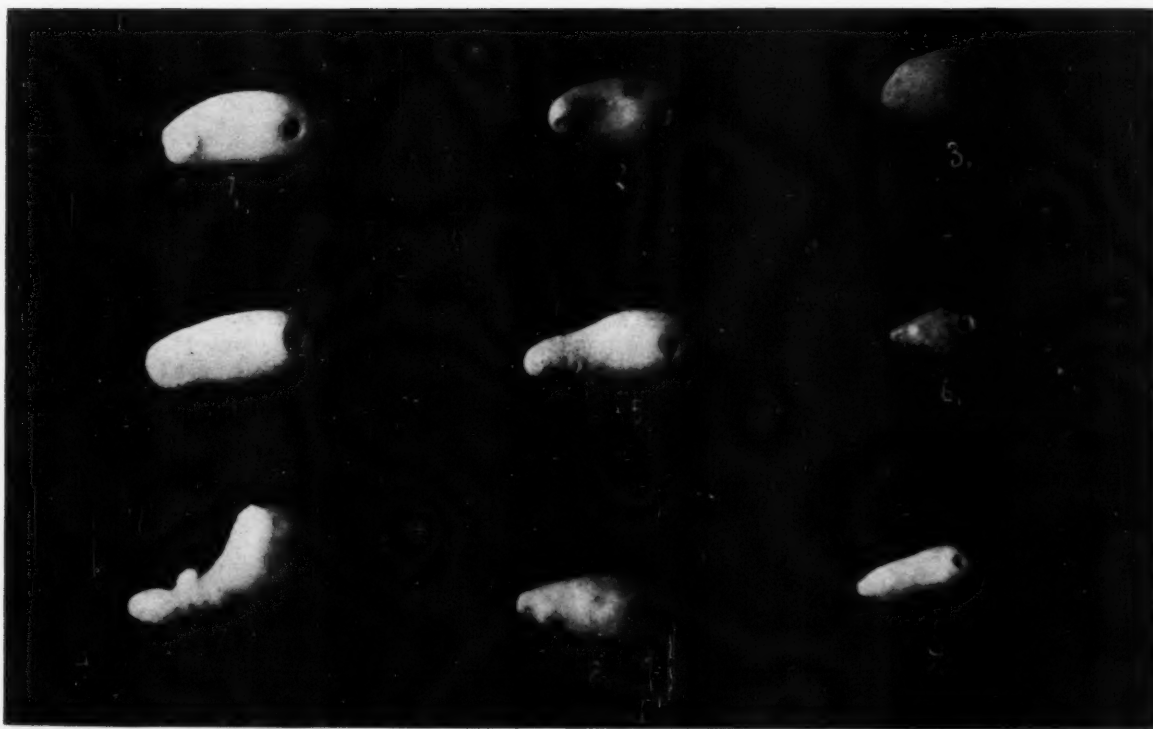


FIG. B

## EXPLANATION OF PLATE VII

Fig. A. *Carmyrius exoporus*, n.sp.  $\times 2\frac{1}{2}$ .

Fig. B. *Stephanopharynx compactus*.  $\times 2\frac{1}{2}$ .

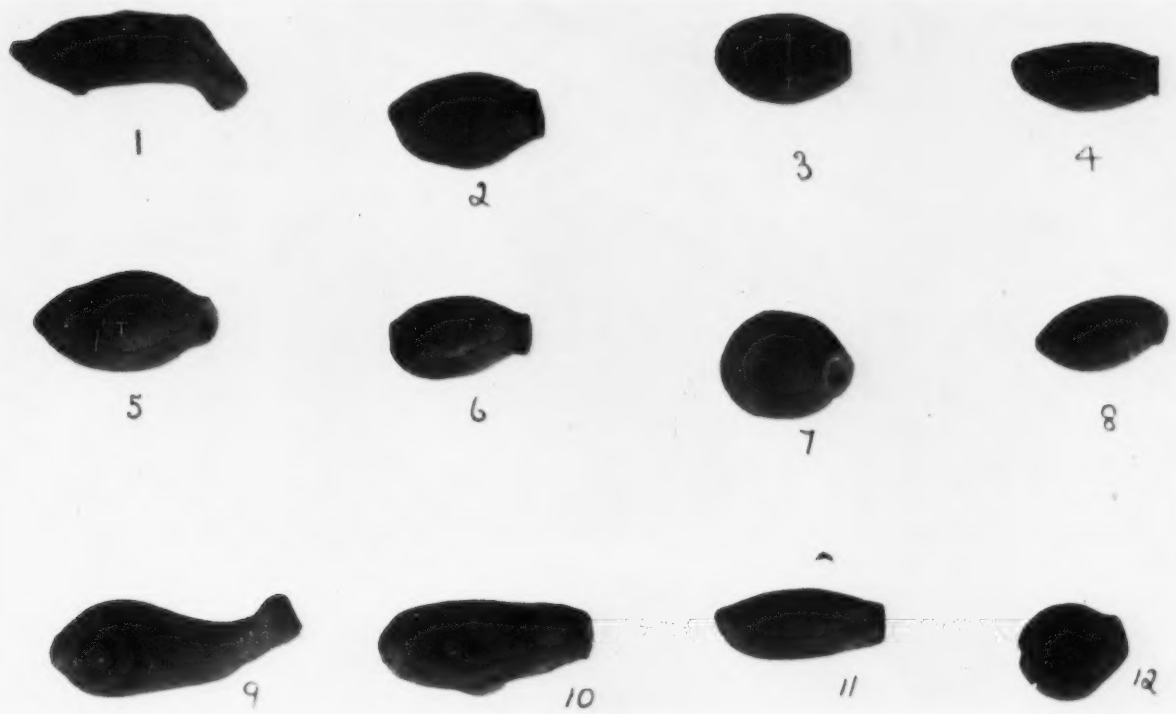


FIG. A

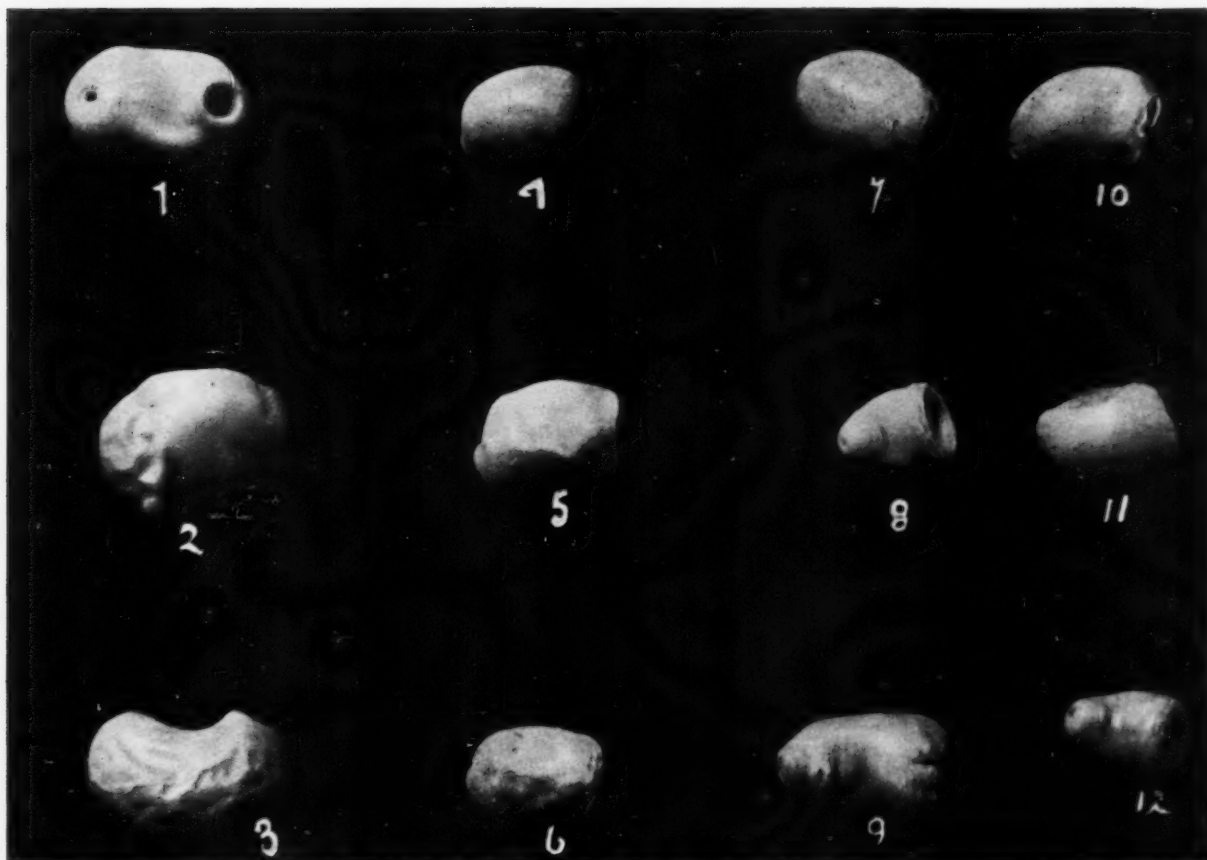


FIG. B

## EXPLANATION OF PLATE VIII

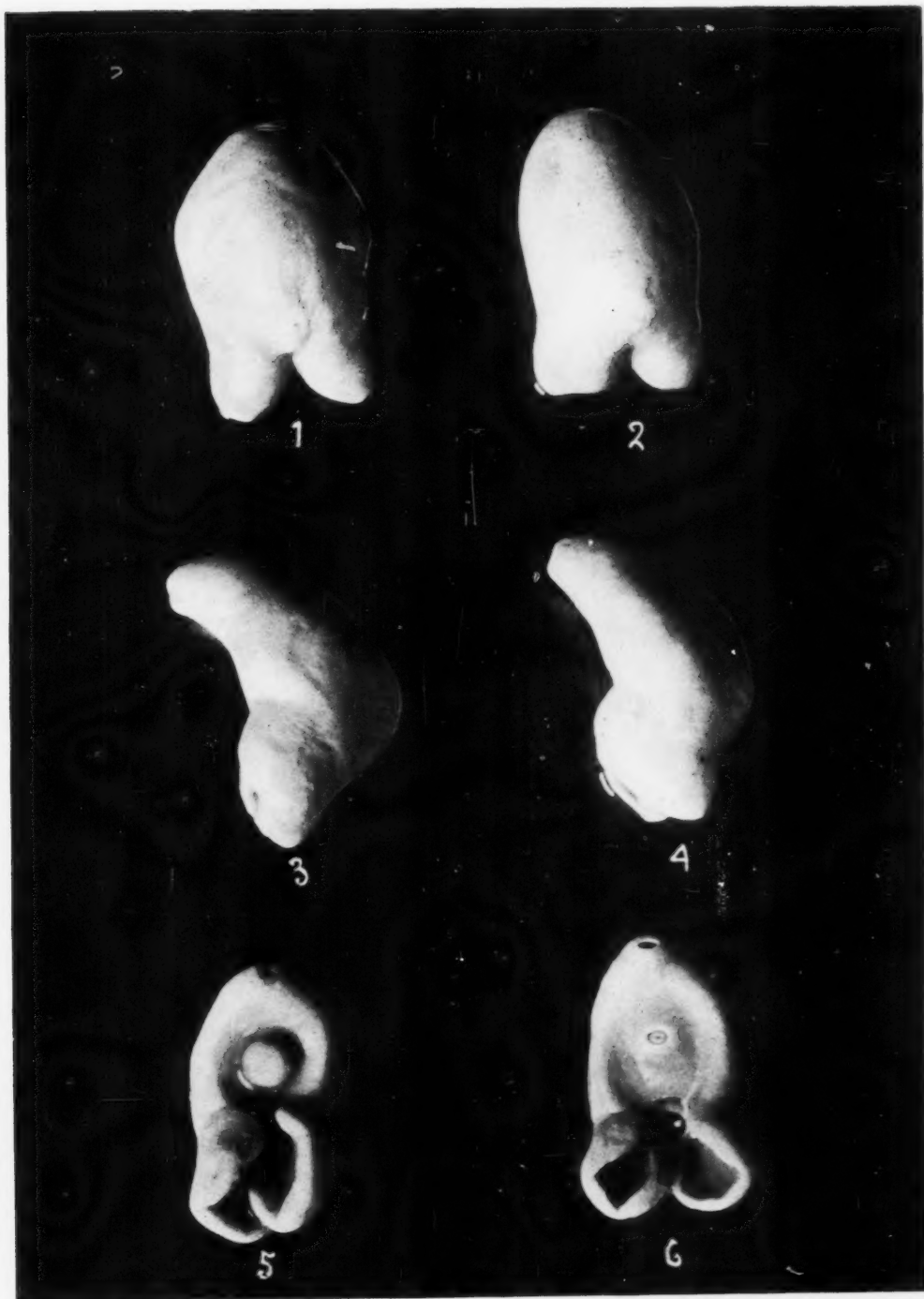
*Brumptia gigas*

Figs. 1 and 2. Dorsal view.

Figs. 3 and 4. Lateral view.

Figs. 5 and 6. Ventral view. (5) Genital papilla extruded; (6) genital papilla retracted.  $\times 2\frac{1}{2}$ .







## MALARIA IN AUSTRALIA

BY

P. A. MAPLESTONE

*(Received for publication 25 February, 1923)*

The following is a short account of the history of malaria in Australia as far as it can be collected from the published records. In compiling this review all the information prior to the year 1912 has been taken from Cleland (1914); since 1912 the original articles in the medical press and the various Government reports have been consulted. This is not a complete discussion of all the references to malaria in Australia; many of the earlier accounts are given by laymen, or are merely the expression of an opinion by a medical man without definite proof, and a considerable number of the later records are purely of local interest, so they have been ignored. In discussing the subject, the various States and the Northern Territory of the Commonwealth are considered separately.

### QUEENSLAND

According to Cleland (1914), the first account of malaria in Australia by a medical man is that of White (1867). In all probability this is the same outbreak as the one to which Elkington (1912) refers, and which he concludes was introduced to Burketown by a ship from Java. Cleland mentions that for some years prior to 1885 there was extensive and severe malaria in North Queensland. In support of this statement he quotes the following authorities, viz.:—A'Hearne (1890) for Townsville, Graham-Browne (1890) for Charters Towers, Hunt (1890) for Hughenden, and James (1891) for Croydon. But from the quotations taken by

Cleland from these authors' writings it is by no means clear that all the epidemics included in this series were due, altogether or even partly, to malaria. For instance, there is no way of finding out if all A'Hearne's cases were malaria; Graham-Browne's description of the Charters Towers epidemic is unlike malaria; Hunt obviously confuses typhoid fever and malaria, and James under the term 'Gulf fever' describes all his febrile cases, some of which were probably malaria. For the same period Jeffris-Turner says he only saw three cases of malaria in children in Brisbane. Whatever these various outbreaks were, it is a striking fact that since the advent of more accurate diagnostic methods, malaria has not been recorded from any of the above towns, but enteric fever is fairly often encountered there.

From Cleland's account it appears that O'Brien (1908) was the first to record finding the malaria parasites in Australia; nearly all his cases were simple tertian, but he writes of finding a few quartan and malignant tertian. O'Brien's observations were apparently made at Yarabah mission station near Cairns. Breinl (1911), without quoting an authority and after only a few months in the country, reported malaria to be epidemic in 'parts of Queensland,' mentioning specifically Innisfail, Cooktown, and Saxby River. Nevertheless, he evidently saw some malaria, as he adds that the locally acquired cases were simple tertian, and that the few cases of malignant tertian that he saw were infected in Papua (=British New Guinea).

Elkington (1912) refers to a localised epidemic of malaria, which occurred at Kidston on the Einasleigh gold field in 1910. There were 120 cases and 24 deaths in a population of 400. The outbreak was investigated by Dr. Baxter-Tyrie, who concluded that the disease had been introduced from New Guinea. From the latter's report Elkington concludes that malignant malaria and blackwater fever are endemic; there is no further reference to this 'endemic' centre in the medical literature nor in the Government reports, therefore it is clear that Elkington's conclusion was premature. Neither is there any evidence that malaria spread from Kidston to any of the surrounding camps.

Although a few cases of malaria undoubtedly occur annually in the coastal districts of North Queensland, there is no way of finding out their numbers. In the Annual Health Report for the State, acute malaria first appears as a notifiable disease in the period 1st July, 1915, to 30th June, 1916. The figures up to the present time are given in Table I.

TABLE I.

Cases of malaria notified in Queensland.

Year							No. of cases				
1915-1916	...	...	...	...	...	...	...	...	...	...	79
1916-1917	...	...	...	...	...	...	...	...	...	...	213
1917-1918	...	...	...	...	...	...	...	...	...	...	72
1918-1919	...	...	...	...	...	...	...	...	...	...	10
1919-1920	...	...	...	...	...	...	...	...	...	...	9
1920-1921	...	...	...	...	...	...	...	...	...	...	9
1921-1922	...	...	...	...	...	...	...	...	...	...	19

Unfortunately there is no way of ascertaining how many of the above cases were contracted in Queensland, and how many came from elsewhere, except that in 1916-1917 the Sanitary Inspector of the Northern District states in his report that 119 cases of malaria occurred in Cairns for that year. It can also be found indirectly, by comparing the above figures with those of the Australian Institute of Tropical Medicine for corresponding periods, that nearly all the remaining cases were returned soldiers who had become infected outside Australia.

Although this information is very incomplete it is quite obvious that the Cairns epidemic was short-lived and that not many cases can be occurring there at the present time. A possible explanation of this short epidemic in Cairns is that this is the first port of call for boats coming from New Guinea to Australia. In the period immediately preceding and during the sudden increase in malaria in this town, large numbers of soldiers returning from New Guinea were calling there, and the majority of them were being sent home because they were suffering from malaria. The extra opportunity for the mosquitoes of Cairns to become infected soon reacted on the local inhabitants; but in 1918, when traffic between New Guinea and Australia returned to normal and fewer persons with malaria parasites in their blood were calling at Cairns, the incidence of malaria there suddenly dropped and has remained low ever since. It is true that Breinl and Taylor (1918), after a malaria and mosquito survey of the town recommended the filling and draining



of various swamps in and around it ; but the drop in malaria incidence cannot be explained in this manner, because Mr. Hill, Entomologist of the Australian Institute, visited Cairns in 1921 and at the writer's request examined the mosquito-breeding places recorded by Breinl and Taylor in 1918. He reported that little had been done in reducing these breeding places.

Dr. H. H. Willis, in a letter to the writer in May, 1921, informed him that while on a 'hookworm' survey of the native settlement on the Palm Islands he had found nine or ten\* cases of acute malaria which he had diagnosed microscopically. Over a month later the writer visited these Islands ; he examined all the natives (over 300) and found that five or six\* of the cases reported by Willis had crescents in their blood. All the other natives were negative on blood examination, no palpable spleens were found although there were numerous children, and no fresh cases had occurred between the visits of Willis and the writer. The evidence of the origin of this small outbreak was not satisfactory, but as far as could be gathered it seemed likely that the malaria had been introduced from the mainland by some recent arrivals. It is remarkable that the outbreak did not spread further, because the natives were living closely congregated in unscreened grass huts, and Hill found *Anopheline* mosquitoes breeding close to the dwellings (see Table VI).

The history of Townsville during recent years from the point of view of malaria is of considerable interest, because since the establishment of the Australian Institute of Tropical Medicine in 1910, more reliable records are available from there than from any other town in Northern Australia. Many parasite carriers have been constantly arriving in Townsville for treatment during the eleven and a half years January, 1910 to June, 1921, and for the whole of that time no case of malaria has ever been discovered which was contracted in the town or its surroundings. There is also abundant evidence that the same species of *Anophelines* are found as in other Coastal towns where malaria occurs. Townsville is well within the tropics and is by far the largest town in Northern Australia with a population of about 25,000, nearly all of whom are whites.

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\* Figures from memory.

## THE NORTHERN TERRITORY

Again consulting Cleland (1914) it is found that Wood (1889) said that malaria was very prevalent in the Northern Territory during the years 1879, 1880, and 1881. Holmes (1913) gives the following figures taken from the official records :—

TABLE II.

Deaths in the Northern Territory.

Year	Total Number of deaths	Deaths due to 'fever'
1879 ... ..	166	61
1880 ... ..	154	61
1881 ... ..	100	51

It was at this time that gold-mining was at its height and there were many mining camps in the country with no medical man near them and no sanitary precautions in force. Although many of the deaths were in all probability due to malaria, it should be borne in mind that a large number of the cases could not have been seen by a medical man, so that the diagnosis of 'fever' on the death certificates is not of much use for accurate record.

The Umbrawarra tin mining field was opened up about the year 1909 and shortly afterwards malaria broke out there. This epidemic was authenticated by Breinl (1912) who gives an account of it. The record is of considerable value, because the epidemic is shown to be due beyond all doubt to malaria, and the conditions at Umbrawarra were, in all probability, identical with those obtaining on mining fields in earlier times where similar epidemics occurred. These rushes to new mineral discoveries attract men from other parts of the world, and all the mining camps of Northern Australia have contained men from New Guinea, which is a highly malarious country. In the case of Umbrawarra, Breinl traced the origin of the outbreak to miners from New Guinea arriving with parasites in their blood; from this fact it seems most likely that the earlier epidemics on other mining fields in the same regions of Australia were due to a like cause. The Umbrawarra epidemic came to an abrupt end, primarily by the departure of the majority of the miners, for in 1913, when the writer visited the field, there were only two miners remaining;

but it is strange that the disease did not spread to Pine Creek, a permanent settlement only thirteen miles distant and which during the height of the activities at Umbrawarra was in daily communication with it, receiving all the men who were seriously ill and many of whom must have had malaria parasites in their blood. The same species of Anopheline has been recorded from both places.

Breinl and Holmes (1915) visited several districts in the Northern Territory, including the Daly and Alligator Rivers, and Bathurst and Melville Islands; they found no signs of malaria among the natives in any of these localities either on blood examination or spleen palpation. In the same report it is mentioned that Holmes in 1912 found four out of twenty natives examined on Melville Island to be suffering from malignant tertian malaria, from which it is clear that within three years the disease had disappeared from the island without any special anti-malarial measures being taken.

The only other published records of malaria in the Northern Territory are those in the Annual Health Reports and in the Annual Reports of the Darwin Hospital. A brief outline of the local conditions will indicate that the figures in Table IV are only very approximate.

The total area of the Northern Territory of Australia is well over 500,000 square miles; there is only one\* medical officer in the whole country and he spends practically the whole of his time in Darwin. For this reason most of the cases of sickness reported, and of deaths registered, are not certified by a qualified man, but are furnished by the local police; consequently the only reliable returns are those for the Darwin Hospital. In the year 1918 the highest population since 1910 was recorded, and this is given in Table III along with the latest figures available.

TABLE III.  
Population of the Northern Territory.

Year	Europeans	Asiatics	Half-castes	Total †
1918    ...    ...    ...    ...    ...	3767	1177	118	5062
1921    ...    ...    ...    ...    ...	2478	1094	?	3572

\* From 1911 to 1915 there were four medical officers in the Northern Territory, and two of them did considerable travelling.

The aborigines consist of numerous small nomadic tribes, hence their numbers cannot be accurately determined. The most reliable estimate of the number of natives that the writer has ever been able to obtain, was given to him about ten years ago by an official who had spent over forty years in the country and who had travelled practically all over it. This officer was of the opinion that there were not more than 30,000 natives in the whole country. Although far from being precise these figures at any rate indicate that it is very thinly populated.

TABLE IV.

Malaria records for the Northern Territory

Year	Total Number of cases reported	Cases treated in Darwin Hospital	Deaths	Remarks
1897...	?	18	7	
1898...	?	8	8	
1899...	?	6	5	
1900...	?	5	6	
1901...	?	1	9	
1902...	?	1	6	
1903...	?	2	6	
1904...	?	12	8	
1905...	?	1	0	
1906...	?	6	0	
1907...	?	12	7	
1908...	?	23	16	
1909...	?	44	18	
1910...	?	27	18	
1911...	?	11	3	
1912...	?	12	0	
1913...	?	6	1	
1914...	1	—	0	
1915...	—	—	—	
1916...	'Prevalent'	?	15	For 18 months ending 30.6.1917
1917...	—	—	—	
1918...	45	?	?	
1919...	—	—	—	Not available
1920...	59	?	?	
1921...	'Many cases'	24	2	

In addition to the above table the following extracts from the Health Reports are appended.

**1912.** Malaria is not as prevalent as it is popularly supposed to be. The only death ascribed to malaria is registered 'kidney troubles and fever.' Practically all deaths outside Darwin are registered by the police, so the accuracy of the diagnosis is extremely doubtful. Malaria



is unknown at Pine Creek and Darwin, the two largest settlements. Some cases of malaria were found among the natives on Melville Island.

**1913.** The single death registered as due to malaria was diagnosed by a layman. Two of the medical officers travelled extensively during this year and only one case of malaria was seen, although this disease was specially looked for, and no cases were found on Melville Island where it was seen the year before.

**1915-1917.** Malaria was 'very prevalent' in several localities, e.g., the Pine Creek railway extension camps and Maranboy mining field. More than 50 per cent. of the cases were only diagnosed clinically, and although it is not stated, it is almost certain that a number of the cases were not seen at all by a medical man. It is considered that malaria is not endemic.

**1918.** One case was contracted in Darwin.

NOTE.—This is the only record of a case contracted in Darwin that the writer can find.

**1920.** All of the 59 cases reported for the year came from the country districts and were of a mild form. Three more serious cases were apparently contracted elsewhere.

**1921.** Many cases have occurred during the past few months, none of which were contracted in Darwin. The increase of the past few years is ascribed to the introduction of returned soldiers with parasites in their blood.

#### NEW SOUTH WALES

Early in the year Jamieson (1915) reported a case of malaria which the evidence showed to have been contracted at Gosford not far from Sydney. Commenting on this case, Cleland (1915) stated that apart from unreliable records in the comparatively early days of settlement he only knew of one other case contracted in the State. This was in a baby a few days after birth, who was born of a mother suffering from malaria at the time; he considers this to be a case of direct infection.

On the 17th March, 1915, 'Acute malaria' was made compulsorily notifiable throughout New South Wales; this regulation continued in force until 28th November, 1919, when it was withdrawn. The annual figures for this period are given in Table V.



TABLE V.

Cases of malaria notified in New South Wales.

Year										Number of cases
1915	...	...	...	...	...	...	...	...	...	105
1916	...	...	...	...	...	...	...	...	...	61
1917	...	...	...	...	...	...	...	...	...	17
1918	...	...	...	...	...	...	...	...	...	11
1919	...	...	...	...	...	...	...	...	...	35

It is not stated in these returns whether any of the cases were locally acquired, and all that can be gathered in this respect is that in 1915 the Chief Health Officer in his letter of presentation of the annual report states, that of the 105 cases recorded in that year, all except 14 were returned soldiers; it is, of course, possible that all of the fourteen cases who were not soldiers also acquired their infections in other countries.

There are two other records of isolated cases which seem beyond doubt to have been contracted in New South Wales; one of these was reported by Evans (1919) at Wyong, and the other by Clayton and Utz (1921) near Tumbarumba. This completes the published record of malaria for New South Wales.

#### VICTORIA

Doyle (1921) reported a case of malaria at St. Arnaud, which was locally acquired. As far as can be ascertained this is the only case of malaria ever recorded in Victoria.

In South Australia and Tasmania there is no evidence that malaria has ever occurred. The North-west of Western Australia which adjoins the Northern Territory is comparable to the latter both in its malaria incidence and conditions of living. No references to malaria in this part of the country can be found in the literature, nor are the Government reports from this State available, so actual figures cannot be given.

The writer had spent altogether upwards of five years in North Queensland and the Northern Territory (see map, places underlined), and during that time he has seen only two cases of malaria contracted in the country, the small outbreak on Palm Island in 1921 excepted. Experience has led him to the conclusion, that the inhabitants of Tropical

Australia are prone to ascribe all their ills to malaria and that this opinion is rarely confirmed by microscopic diagnosis.

The tendency of the layman to exaggerate the incidence of malaria reacts on the police, who in the absence of medical assistance are inclined to register all deaths not clearly due to violence as due to malaria. These figures are given in the annual health reports and so the popular and erroneous opinion of the prevalence of malaria is to some extent supported in official returns.

#### ANOPHELINE MOSQUITOES FOUND IN AUSTRALIA

According to Ferguson (1921) only five species of Anophelines have ever been recorded in Australia. They are:—

1. *A. corethroides*, Theobald, 1907.
2. *A. (Pyrethrophorus) atratipes*, Skuse, 1888.
3. *A. (Pyrethrophorus) stigmaticus*, Skuse, 1888.
4. *A. (Nyssorhynchus) annulipes*, Walker, 1850.\*
5. *A. (Myzorrhynchus) barbirostris*, de Wulp, var. *bancrofti*, Giles, 1902.

*A. corethroides* is only found in South Queensland.

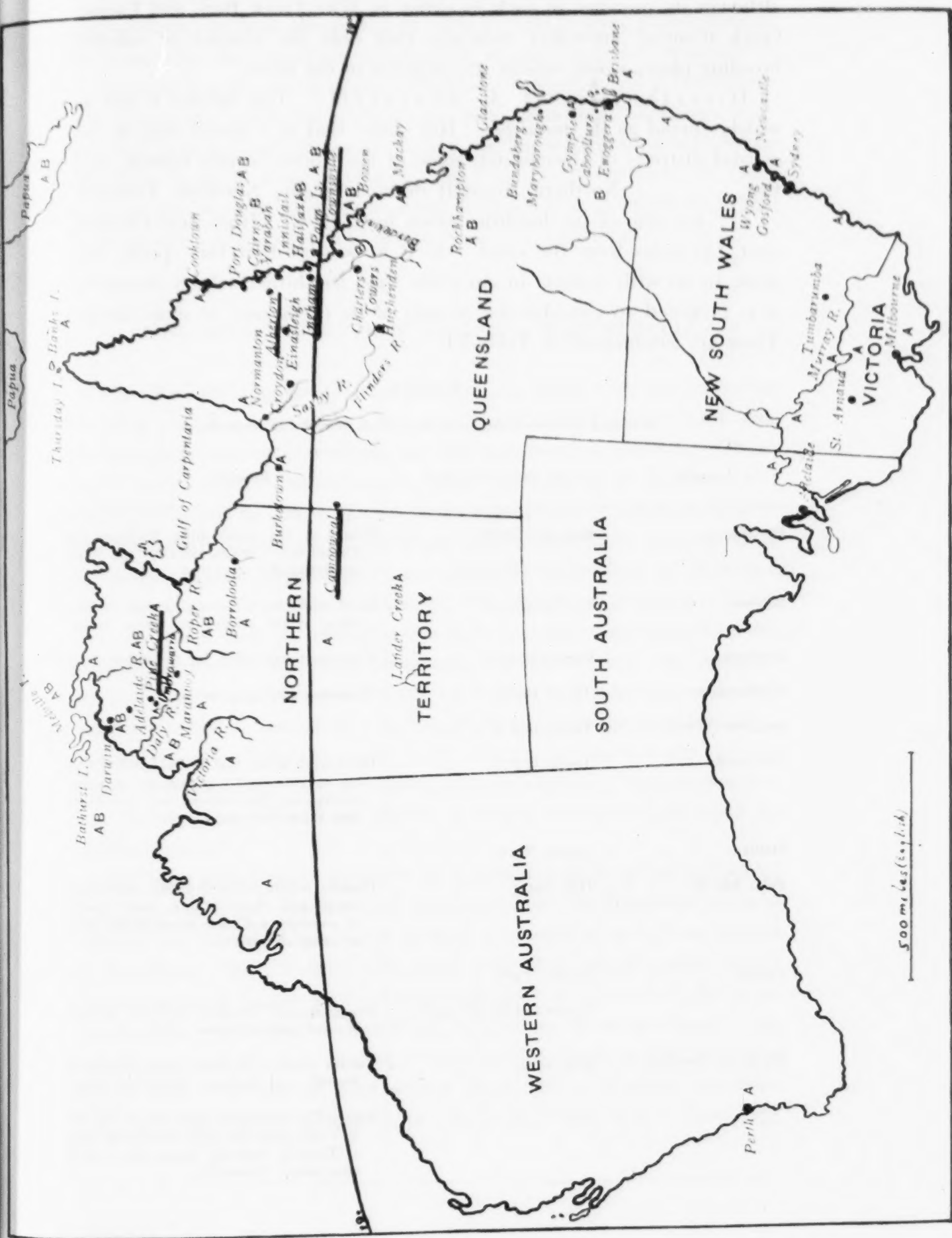
*A. atratipes* was recorded by Bancroft (1908) from South Queensland and it was also recorded earlier by Skuse at Berowra, New South Wales.

*A. stigmaticus* has been recorded only once from a single locality in New South Wales.

These three species are only found in parts of Australia where malaria does not occur and are so restricted in distribution that they cannot have any bearing on the spread of this disease at the present time. The other two species, viz., *A. annulipes* and *A. bancrofti* are much more widely spread and as, from circumstantial evidence which is all that is available, they seem to be connected with malaria, their occurrence will be considered in more detail.

**Distribution of *A. annulipes*.** Hill (1922) summarises the distribution of this species as follows:—'*A. annulipes* is undoubtedly the most widely-distributed Anopheline found in Australia, having been recorded from Tasmania northwards to Banks Island (Torres Strait), and from South Australia, Central Australia, Northern Territory, South-west Australia, and North-west Australia. It is most probable that it does not occur in the elevated districts of South Australia and North Queensland (Atherton Tableland), and possibly not in some of the arid inland districts,

\* Until recently, another species *Anopheles amictus* Edwards, 1921, has been confused with *A. annulipes*. *A. amictus* occurs at Townsville, Palm Island and Port Darwin. [Editors.]



500 miles (800 km)

although its presence in such localities as Wire Creek Bore and Lander Creek (Central Australia), indicates that only the absence of suitable breeding places would inhibit its existence in the latter.'

**Distribution of *A. bancrofti*.** This species is not so widely spread as *A. annulipes*. Hill states that it is found only in the coastal districts of Queensland, some of the Torres Straits Islands, and the ' . . . Northern (coastal) districts of the Northern Territory . . . ' but one of the localities given by Hill, viz., Horseshoe Creek is over 150 miles from the coast. As it is possible that this species has more to do with malaria in Australia than has hitherto been supposed, it is proposed to consider the records of its occurrence in more detail. These are summarised in Table VI.

TABLE VI.

Principal records of the occurrence of *A. bancrofti* in Australia.

Locality	By whom recorded	Remarks
Brisbane ... ..	Bancroft (1908) ... ..	Found in the scrub from Enoggera to Caboolture; males, larvae, and eggs never found.
Brisbane ... ..	Cooling (1913) ... ..	Once taken in a house; a few females taken in the scrub; no larvae found.
Brisbane ... ..	Cooling (1914) ... ..	Four specimens taken for the whole year.
Rockhampton ... ..	Taylor (1916) ... ..	Numerous adults, no larvae.
Burdekin River ... ..	Taylor (1913) ... ..	
Townsville ... ..	Taylor (1913) ... ..	Hill (1922), writes that he has failed to find the species over a period of 2½ years' continuous observation and concludes that it has died out.
Halifax ... ..	Taylor (1916) ... ..	
Palm Islands ... ..	Hill (1922) ... ..	Plentiful within 200 yards of No 2 aboriginal camp and close behind main camp. <i>A. annulipes</i> not taken nearer than ¼ mile of camp clearing.
Cairns ... ..	Taylor (1916) ... ..	
	Taylor and Breinl (1918) ... ..	Numerous, and breeding freely in swamps in and around town.
Northern Territory ... ..	Hill (1922) ... ..	Twelve distinct localities given, including Melville and Bathurst Island (see map).
		<i>Note.</i> —The mosquito observations in the N.T. are more thorough than in any part of Tropical Australia except for a small area round Townsville.



With the exception of Brisbane, Townsville, Cairns and to some extent the Northern Territory, the records in Table VI refer to a single observation. It is probable that more extended work would reveal the presence of *A. bancrofti* in many other parts of Northern Australia, but it is unlikely that this species exists far south of Brisbane, because it has never been recorded in New South Wales at all, and the knowledge of mosquitoes in this State is much more advanced than it is in Queensland. In fact, as far as the records go, there is some evidence that *A. bancrofti* is not found in any numbers south of about 19° South Latitude.

### THE INSECT VECTOR OF MALARIA IN AUSTRALIA

*The Medical Journal of Australia* has on more than one occasion (Leading articles, 1915, p. 171 and 1921, p. 512, etc.), pointed out that the mosquito carrier of malaria has not yet been determined. Breinl (1912) stated that *A. annulipes* was the probable vector at Umbrawarra; in support of this he quotes the successful experiment of Kinoshita (1906), who successfully infected *A. annulipes* with *Plasmodium falciparum* in Formosa. A little later Breinl (1914) definitely stated that *A. annulipes* was the carrier of malaria in Australia, but quoted no authority. Since these two references *A. annulipes* has been frequently mentioned in the medical literature of Australia, being variously described as 'the probable carrier,' 'the presumed carrier,' 'the carrier,' etc., but in most cases no authority is given, and when it is, Kinoshita (1906) is the only reference. The statement by Harrison (1922) is an accurate summary of the present state of our knowledge in this respect, when he says that there is evidence that the local Anophelines are capable of acting as intermediate hosts for malaria parasites.

The only record of *A. annulipes* as a malaria carrier given by Chanal (1921), is the single experimental result obtained by Kinoshita already referred to; Chanal's conclusion is that *A. annulipes* should be classed as dangerous. But it should be noted that although Kinoshita states that he infected 60 per cent. of his mosquitoes, a detailed study of his experiments shows that he used the species on three occasions. The first time, out of five fed, all died within three days, the second time nine insects were used and all died within three days of feeding, the third time eight mosquitoes were used, of which three died within three days



and three of the remaining five became infected. It is this result Kinoshita gives as 60 per cent. positive ; only *P. falciparum* was used.

*A. bancrofti* seems by common consent to have been almost completely ignored as a possible malaria carrier in Australia, for the only references to this species in this connection are the following, viz. :—Cleland (1910) includes it in a list of the then known malaria carriers. It is next mentioned by Cooling (1914) who suggests that it may be a malaria carrier, because Stephens and Christophers (1902) were successful in infecting *A. barbirostris* in the laboratory in India. Since that date, according to Chanal (1921), *A. barbirostris* has been found in nature and infected in the laboratory both with *P. falciparum* and *P. vivax* on several occasions in various parts of the Malay Archipelago ; but as all these records refer to a different variety of the species which does not occur in Australia, they have no bearing on the subject. One other reference to *A. bancrofti* as a malaria carrier is made by Breinl (1915) who, in an article on New Guinea, states that *Nyssorhynchus bancrofti*\* is not a malaria carrier. This statement is not supported by any evidence.

With regard to the distribution of Anophelines in Australia, Breinl (1914) says, ' . . . The distribution of malaria in Australia corresponds, on the whole, with the incidence of the mosquito *Nyssorhynchus annulipes* . . . It is curious to note that there are localities where the mosquito has been found, but where malaria is practically non-existent.'

Again, Breinl and Taylor (1918) remark, ' . . . *Nyssorhynchus annulipes* which, judged by its distribution in relation to malarial infested regions in Northern Australia, most probably acts as a malaria carrier . . . '

These two statements may be more or less correct as far as they go, but they do not explain why malaria is practically never found far south of Cairns, whereas *A. annulipes* is spread all over Australia. If the explanation of the restriction of malaria to Northern Australia is to be found in the distribution of a special Anopheline, it will be found that the occurrence of *A. bancrofti* much more nearly corresponds with the malaria distribution than does *A. annulipes*. *A. bancrofti*, however, also exhibits one or two striking exceptions to the rule, so it is considered that the peculiar distribution of malaria in Australia is due to other causes, not yet ascertained.

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\* *Myzorbynchus bancrofti* is obviously the species intended, for Taylor (1914) in the list of mosquitoes taken by Breinl on this expedition includes it, and as far as the writer can ascertain there is no such species as *Nyssorbynchus bancrofti*.

As far as the writer can find out, the only explanation that has ever been offered as to why malaria fails to become established in the greater part of Australia is the mathematical hypothesis of Ross (1910); all the authors who mention this subject are of the opinion that there are too few mosquitoes or too few susceptible human beings in most parts of Australia. It is unlikely this is the sole reason, if it is the reason even in part, for it is by no means in the most thickly populated parts of Australia where *Anopheline* mosquitoes are found that malaria outbreaks occur.

There is another set of conditions which seem to the writer worthy of consideration, and which have never been considered, and that is the relation between malaria outbreaks and meteorological records. Gill (1920 and 1921a) has studied the incidence of malaria in parts of India along with the mean temperature and relative humidity readings, and the same author (1921b) extended his observations to England. As a result of this work he considers it probable, that before malaria is able to spread in a locality it is necessary to have a monthly minimum mean temperature of  $61^{\circ}$  F. and a minimum mean relative humidity of 63 per cent. At the same time he points out this is not yet conclusively proved. In this connection it is worth noting that in Kinoshita's successful experiment with *A. annulipes* the temperature remained between  $28^{\circ}$  and  $30^{\circ}$  C. the whole time, and in his conclusion he states that complete development of the oocysts of *P. falciparum* cannot take place in this mosquito except in a high and unvarying temperature.

### SUMMARY

As far as can be gathered from the incomplete and unreliable records available, malaria is only mildly endemic in Australia north of  $19^{\circ}$  South Latitude. *A. annulipes* and *A. bancrofti* the only two possible malaria carriers in Australia under present conditions are much more widely distributed than is malaria.

In various localities north of  $19^{\circ}$  South Latitude small epidemics of malaria occur from time to time; these outbreaks are of short duration, their origin is generally traceable to the introduction of malaria carriers from abroad, the disease does not spread to adjoining camps and towns, and soon dies out, without any very active anti-malarial measures being instituted.

The scarcity of population and Anopheline mosquitoes is not a satisfactory explanation of the absence of malaria from the greater part of Australia.

It is of the first importance to discover the mosquito carriers of malaria in Australia, and when this has been done, work along the lines of Gill in India and England would possibly yield interesting and valuable results.

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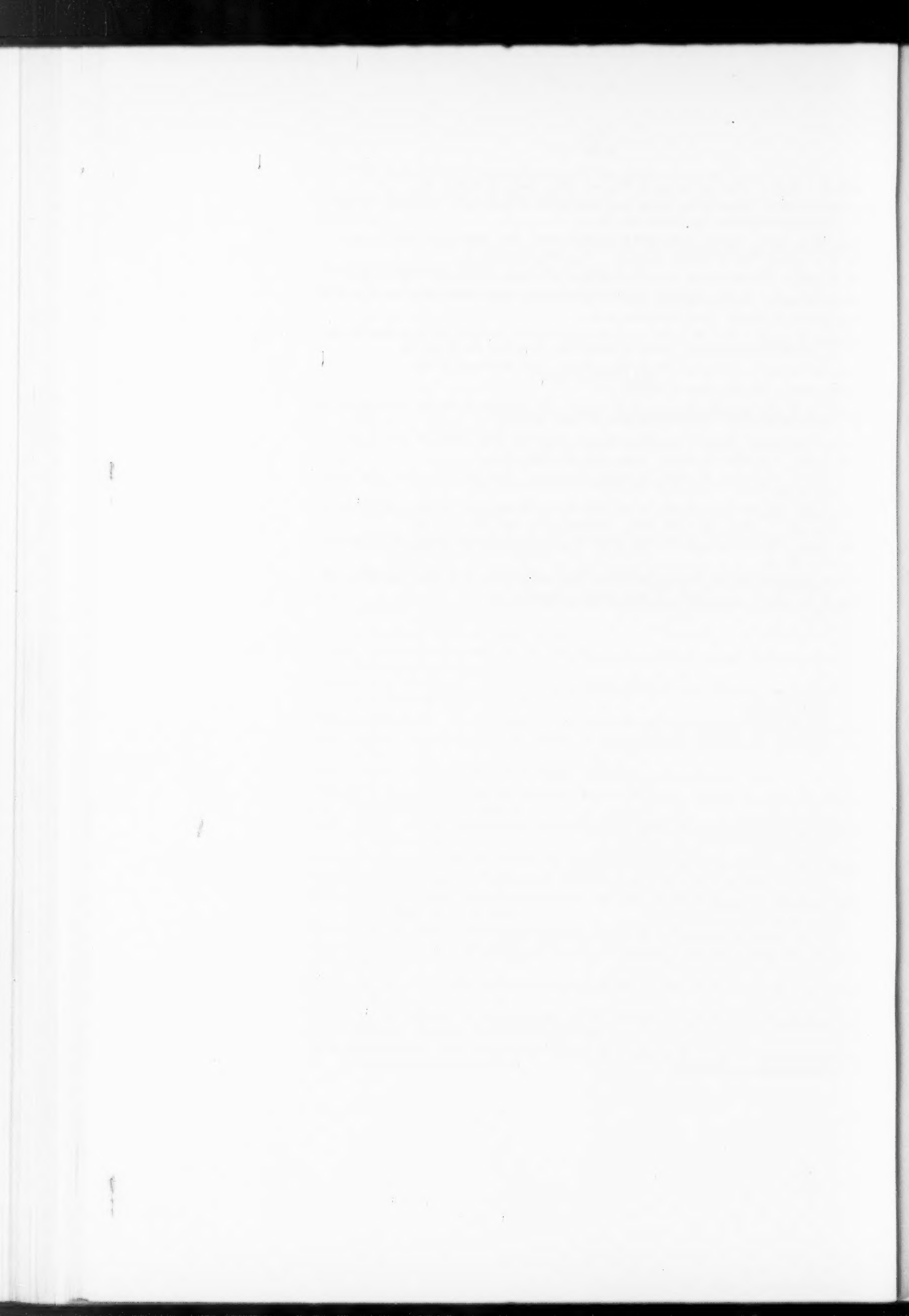
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# COCCIDIOSIS OF CATS AND DOGS AND THE STATUS OF THE *ISOSPORA* OF MAN

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PLATES IX-XIV.

Our knowledge of the coccidia of dogs and cats commences with certain observations recorded by Finck (1854) on the changes undergone by the intestinal epithelium of cats during the process of food absorption. From that time to the present day it has been generally assumed that these animals harbour only one coccidium, which has been usually described during recent years under the name *Isospora bigemina* (Stiles, 1891). Though the measurements of the oocysts given by various observers who have studied the coccidia of these animals have differed considerably, the view that only one form exists has been rigidly adhered to with few exceptions. Perroncito (1882) appears to be the first to have considered it possible that more than one form occurred in these animals, for he separates the one described by Grassi (1879) from that recorded by Rivolta (1874-1878), while Neumann (1888), Railliet (1895) and Neveu-Lemaire (1912) seem to have held the same view. Dobell (1919, p. 177) states that there is no really conclusive evidence to prove that the *Isospora* of the cat is the same as that of the dog, or that both are merely varieties of one species, but he refers to all the coccidia of these animals by the name *Isospora bigemina*, pointing out, however, that Grassi's name *Coccidium Rivolta* has priority over that of Stiles. Reichenow (1921) definitely asserts that the form in the dog is probably distinct from that in the cat, while Nöller (1921), without giving any details, writes of a small and a large form in the cat.

Observations which have been made by the writer during the

past twelve months reveal the fact that there are at least three species of *Isospora* in these animals in England. One of these has an oocyst about 12-15 microns in length, another an oocyst about 25-30 microns in length, and a third an oocyst about 40-45 microns in length. The text-fig. 1 shows the relative size and appearance of the three types compared with the one discovered in man during the war. If these dimensions are kept in mind, the different accounts which have been given by various observers become at once intelligible, and it is possible to identify with some degree of certainty which form was actually under observation. All three have been previously recorded in the literature. In addition to the species of *Isospora*, dogs harbour an *Eimeria* with which we are not for the moment concerned.

#### HISTORICAL REVIEW OF LITERATURE

The first description of a coccidium of the cat was published by Finck (1854). His paper is difficult to obtain, but fortunately Davaine (1860) quotes in full the passage dealing with the bodies observed by this author. As it is of such importance from the present point of view it is quoted *in extenso* from Davaine, pp. 259-260.

"Sur le même animal (le chat) nous avons rencontré une autre forme bien plus singulière (fig. 22). Beaucoup de villosités, semblables du reste à celles chargées de graisse, à la place de gouttes graisseuses, renfermaient, en quantité considérable, des *corpuscules* que nous appellerons *gémisés*, parce que le plus souvent ils étaient réunis par paires. Tantôt une seule et même villosité offrait à la fois et des gouttes huileuses manifestes et des *corpuscules gémisés*, le tout entremêlé d'une manière irrégulière; tantôt les *corpuscules gémisés* remplissaient seuls le bout de la villosité. Ils étaient pour la plupart elliptiques, et leur grand diamètre atteignait à peine un centième de millimètre; la plupart mesuraient  $0^{\text{mm}}, 08$  sur  $0^{\text{mm}}, 07$ , ou bien  $0^{\text{mm}}, 1$  sur  $0^{\text{mm}}, 09$ . Leur contour était fin, net, très noir; leur contenu variable, occupant tantôt presque toute la cellule, plus souvent accumulé vers son centre. C'était une matière granuleuse réunie en une ou plusieurs masses. Il nous a semblé parfois voir une enveloppe commune pour deux corps gémisés.

"Quel est la nature de ces corps? Remak représente un corpuscule semblable au premier aspect, seulement plus grand et non *gémisé*. Il croit devoir le considérer comme un parasite particulier qui se développerait dans les cylindres épithéliaux des glandes de Lieberkühn et dans ceux des conduits biliaires. Il cite Hake et Nasse comme ayant trouvé des formes semblables, par masses, dans le foie du lapin. Kölliker a observé la même chose. Selon lui, les corpuscules du foie du lapin seraient des oeufs de bothriocéphale; ceux des villosités du même animal, plus petits que les premiers, des oeufs d'entozoaires, siégeant dans l'intérieur des villosités et peut-être aussi dans les cellules épithéliales distendues. Dans ce

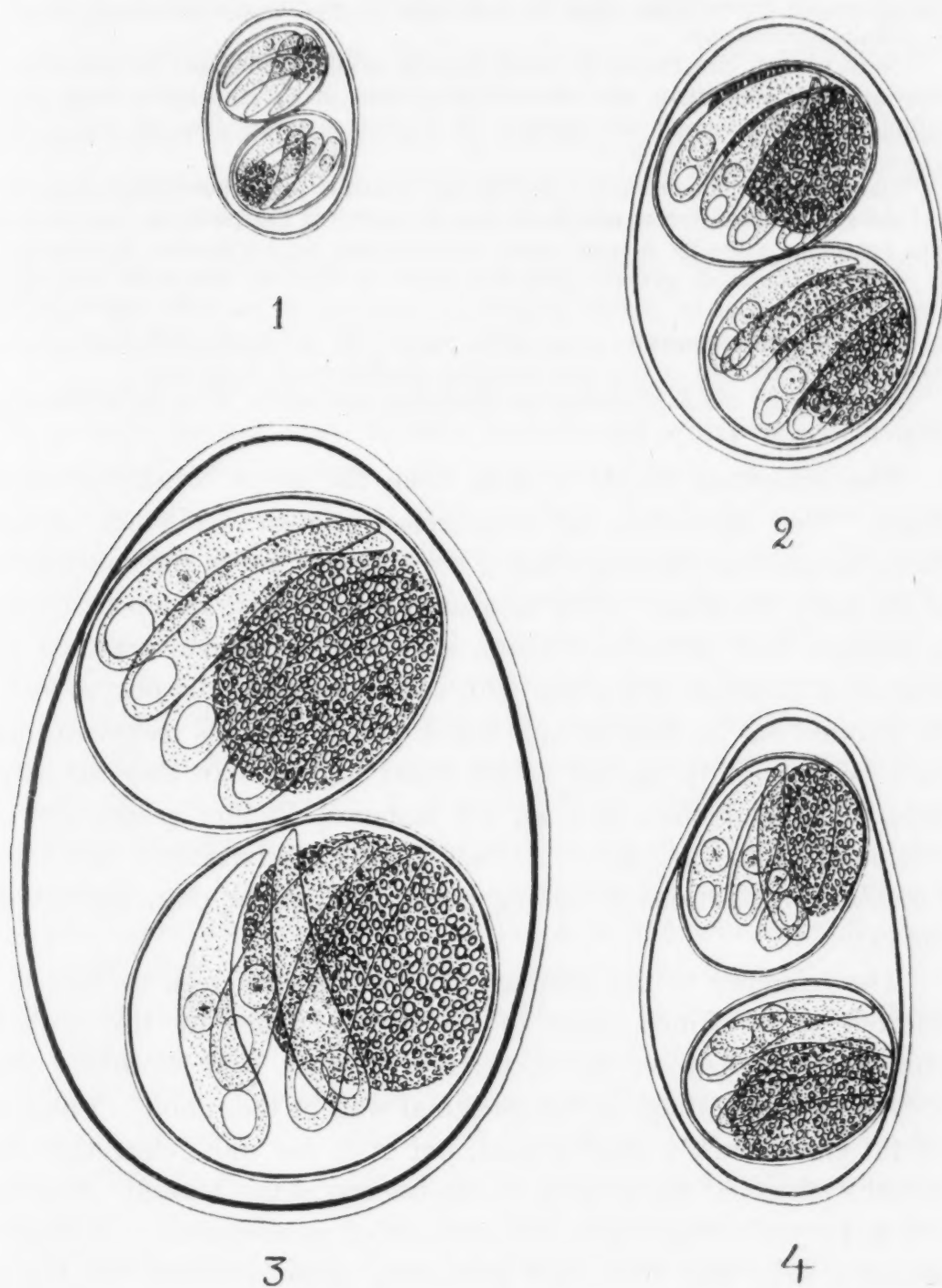


FIG. 1. Diagram of the oocysts of the *Isospora* of cats, dogs and men.  $\times 2000$ .

1. Oocyst of the small form which occurs in the deeper tissues of the villi of cats and dogs and man (*Isospora bigemina* and *Isospora hominis*).
2. Oocyst of the intermediate sized form which occurs in the epithelium of the villi of cats and dogs (*Isospora rivolta*).
3. Oocyst of the large form which occurs in the epithelium of the villi of cats and dogs (*Isospora felis*).
4. Oocyst of the large form which probably occurs in the epithelium of the villi of man (*Isospora belli*).

dernier cas, ils ressemblent, selon lui, à des grosses gouttes graisseuses remplissant les cellules épithéliales.

"Nous n'avons rien trouvé de pareil dans les cellules épithéliales de notre chat ; mais son foie renfermait des amas d'entozoaires plats, elliptiques, long d'un millimètre, probablement des douves. Ils étaient contenus dans des espèces de kystes.

"Quant à nous, tenant compte de l'énorme quantité des corpuscules en question, de l'absence de toute forme semblable dans la cavité de l'intestin, de leur absence dans toutes les villosités n'ayant point subi l'espèce de macération caractérisant les villosités farcies de globules graisseux, enfin de certaines formes de transition entre ces derniers et les *globules géminés*, nous croyons ne pas trop nous hasarder en rattachant les corpuscules en question au fait du mécanisme de l'absorption graisseuse. C'est tout ce que nous pouvons en dire quant à présent."

(Henri Finck : *Sur la physiologie de l'épithélium intestinal*. Thèse de Strasbourg, 1854, 2<sup>e</sup> série, n° 324, p. 17).

The important points to note from the above description are these. The sporocysts or *corpuscules géminés*, as Finck styled them, occurred in the substance of the villi and not in the epithelium of the cat's intestine. They measured 8 by 7 microns up to 10 by 9 microns, had definite contours, and were sometimes enclosed in pairs in a common membrane which was evidently the oocyst wall. As pointed out by Railliet and Lucet (1891), Finck's measurements have been wrongly quoted as ten times higher than they actually were by several observers, as, for instance, Pfeiffer (1890, 1891), Neumann (1892, p. 467). Dobell (1919) inadvertently refers to Finck's investigations as having been made on the dog, instead of the cat.

The reference to the similar but larger bodies seen by Remak, referred to by Finck, have to do with a paper by this author published in 1845 on the occurrence of what were evidently the oocysts of a coccidium in the intestinal wall of the rabbit. Vulpian (1858) cites Finck's observations, but it is not quite clear that he actually observed the oocysts of the cat coccidium himself. Rivolta (1873, p. 382), referring to the presence of psorosperms in domestic animals, says that they had previously been observed by Finck (1854), and also by Ercolani in 1859, in the cat. Perroncito (1882) also quotes Ercolani as having made this observation. Virchow (1860, p. 342 and p. 527) was the next observer to give any details of their structure, though, like Finck, he regarded them as products of fat absorption. He noted that the villi of the greater part of the intestine of a dog were infiltrated with psorosperms. They were on the surface of the intestine, but a larger number were free in the



intestinal contents. They occurred in the interior of the villi and were relatively small and regularly arranged in pairs enclosed by a double contoured membrane. He says they were evidently similar to the paired bodies described by Finck from the cat. He records and figures the oocysts of a coccidium which he found in the kidney of a bat, and which he regarded as similar to the one seen by him in the dog. The parasite of the bat evidently belongs to the genus *Isospora*.

Leuckart (1860, p. 11, and 1866, p. 21) mentions the fact that the intestinal mucosa of a dog which had been used for experiments with *Trichina* was much altered, and covered with a layer of small, egg-shaped psorosperms. He gives no details of their size or structure. He again (1863) refers to them, but is inclined to regard them as metamorphosis products in the intestinal wall. Another reference to these bodies found in another dog by the same author (1879, p. 282) gives no further information, but he was aware of Finck's work and evidently regarded the structures he had encountered as the same as those seen by Finck. He now describes the condition as due to an accumulation of parasites in the villi.

Rivolta (1874) gave a description of certain oviform cells (*cellule oviformi*) which he had found in the intestinal villi of dogs and cats. In his account, which deals entirely with those seen in dogs, he says they had walls showing a double contour, and varied in length from 8 to 12 or even 15 microns; while in breadth they measured 8 microns. The contents of some of the oviform cells are described as being granular and in the form of a nucleus, or as an elongated body like an embryo with granular material at its centre. In some, however, it is stated that in addition to a granular nucleus there were distinctly three or four elongated corpuscles somewhat irregular in shape. There are four figures accompanying this description, and two of these show quite clearly the granular mass and four small ovoid bodies. The oviform cells are described as occurring in the tissues of the villi especially near their tips and not in the epithelium. As evidence of this, a case is quoted where they were present in the villi of a dead animal which, owing to cadaveric changes, had lost its intestinal epithelium entirely. These oviform cells are again described by Rivolta (1877). In this paper mention is made of Finck's observations, and it is pointed out that invasion



by the cells produces grave alterations in the structure of the villi. In a further communication, Rivolta (1877a) states that he has found other cases of the infection in dogs. Examination of the oviform cells in Müller's solution showed that they constantly contained four long corpuscles with rounded ends. Two other stages are described and figured showing the bodies filled with a granular mass which may have indications of a central constriction. He ventures the suggestion that proliferation into two is taking place. The figures show this clearly. The length of the bodies is given as 13 to 16 microns, and the breadth as 12 microns. The statement is made that they are identical with the *corpuscules géminés* observed by Finck. Rivolta compares them with the psorosperms of the liver of the rabbit, and points out that they differ from these psorosperms in that they do not occur in the epithelium, and that segmentation takes place in the body of the host. He sums up his description by stating that there occur two types of these oviform cells. In one type the contents consist of a nucleus with four elongate corpuscles, while in the other there is a large granular nucleus which at times is in process of segmentation. In a later paper, Rivolta (1878) attempts to classify the psorosperms and gregarines of animals. He names the oviform cells of the dog and cat *Cytospermium villorum intestinalium canis*. He again states that two types of this parasite occur. The first varied in length from 8 to 12 microns, and had a breadth of 8 microns. Within was a single elongate granular body like an embryo. After a few days in water there developed three corpuscles and a granular nucleus. The second type was larger, and varied in length from 12 to 16 microns, and had a breadth of about 12 microns. The contents consisted of a single large granular mass which sometimes showed signs of segmentation.

From the above summary of Rivolta's descriptions it is clear that the larger type is the oocyst and the smaller one the sporocyst. He correctly observed the division of the granular mass into two sporoblasts, but did not realise that each of these gave rise to one of the smaller types which are sporocysts. It is evident that the wall of the oocyst was not very resistant, and easily liberated the sporocysts. In his earlier papers he correctly noted and figured the four sporozoites and the residual body within the sporocysts. It

is evident that the infection was limited to the internal tissues of the villi, and did not occur in the epithelium. The size of the oocyst was 12 to 16 microns by 12 microns, and that of the sporocyst 8 to 12 microns by 8 microns. The development was often completed before the oocysts had left the tissues. Incompletely developed sporocysts continued their development in water.

In his book, already noted above, Leuckart (1879, p. 282) discusses the changes produced in the intestinal wall by coccidia generally. He says he has seen these parasites in both dogs and cats. In the latter animals he states that they occur in the epithelium, where complete development takes place. In the case of the dog, they were in the villi, and he evidently regarded them as similar to the structures seen in this situation by Finck, but is doubtful about those described by Rivolta. As regards the cat, Leuckart is the only observer to refer to the complete development of the oocyst in the epithelium. Finck and Rivolta, together with Railliet and Lucet and Stiles, whose observations are considered below, all state that this takes place in the deeper tissues of the villi. Leuckart's account is not always clear as to the actual animal he is referring to, but the statement quoted definitely refers to the cat. As will be explained below, the oocysts of coccidia which develop in the epithelium do not commence to develop till they have left the body, so that Leuckart's statement is difficult to understand. It is possible that oocysts of the larger forms in the epithelium might develop in animals which had been dead for some considerable time, or that Leuckart actually observed the oocysts of the small form in an unusual situation in the epithelium. On the other hand, he may have seen both a large and a small form in these animals, and confused the two. It seems impossible to be certain of the form he refers to in the cat, but his statements about the one in the dog are much more precise.

The next observer to make a contribution to the subject from personal observations was Grassi (1879), who gives a brief account of a coccidium which he calls *Coccidium Rivolta*, from the intestine of the cat. The oocyst is described as giving rise to two spores, each of which contains four germs. In later papers (1882, 1883) he gives under the same name a more detailed description. The oocyst is said to be elliptical in shape with one end more pointed

than the other. At the pointed end there could be detected a sort of spiracle or micropyle. The measurements of the oocyst are given as 30.8 to 27 microns by 24 to 22 microns. Within it is a sphere varying in diameter from 10 to 20 microns, with a central clear area or nucleus. The sphere divides into two daughter spheres, each having a diameter of 14.3 microns. Two sporocysts result, within which are found four embryos and a large residual body. It is important to note that the parasite is described as occurring in the epithelium of the intestine. The description is accompanied by figures which illustrate clearly the structure of the oocysts. From Grassi's account there can be no doubt that he was dealing with a coccidium which was entirely distinct from that described by Finck, Virchow and Rivolta. As pointed out above, this distinction was recognised by Perroncito (1882) and others.

Pachinger (1887) states that he had seen a sporozoon in the oesophagus, stomach and whole length of the intestine of the domestic cat, and that he had encountered a similar form in the kidney of the dog. He says that it belonged to the monospore coccidia with four sickle-shaped bodies. It is probable he was observing the sporocysts of an *Isospora* of the cat, but there are no means of identifying it with certainty, as no measurements were given. The structures he records from the kidney of the dog are quite unidentifiable.

Railliet and Lucet (1888) published an account of oviform bodies which they found in the villi of a dog. They noted their occurrence in pairs, and remarked on their resemblance to the bodies described by Virchow and Rivolta. On account of their association in pairs, they hesitated to pronounce an opinion as to their coccidial nature. After further study, Railliet and Lucet (1890) gave a brief but clear description of these bodies as coccidia which they had observed in the pole cat as well as the dog. In the dog the oocysts are said to vary in length from 12 to 15 microns, and in breadth from 7 to 9 microns. The contents of each divide into two masses, and each of these gives rise to four spores. The fully developed oocysts may occur in the fresh villi, but usually complete development does not take place till they have been in water for a few days. The similar form with oocyst, measuring 8 to 12 microns by 6 to 8 microns, discovered in the pole cat (*Mustela putorius*) occurs in the deeper tissues of the villus.

Stiles (1891) refers to the work of Railliet and Lucet, and says that he has seen the cysts in the villi of dogs. He noted that each might contain a single large mass of cytoplasm or two separate masses suggesting a division into two of the large mass. Stiles gives the name *Coccidium bigeminum* to this parasite. Railliet and Lucet (1891), in a further communication on the subject, accept the name *Coccidium bigeminum* given by Stiles. They refer to the work of Rivolta and Finck, and say there is no doubt that these observers had studied the same organism. They now describe three varieties of the parasite as occurring in the dog, cat and pole cat, which they regard as varieties of *Coccidium bigeminum* owing to differences in the size of the oocysts:—

*Coccidium bigeminum* var. *canis* 12–15 × 7–9 microns.

*Coccidium bigeminum* var. *cati* 8–10 × 7–9 „

*Coccidium bigeminum* var. *putori* 8–12 × 6–8 „

As pointed out by Wasielewski (1904) these variations in size are insufficient to justify a separation of varieties on this basis alone.

The papers by Railliet and Lucet are not illustrated, but a figure by Railliet appears in the English translation of Neumann's work on 'Animal Parasites' (1892, p. 437). This figure again appears in the second edition of the *Traité de Zoologie Médicale et Agricole* by Railliet (1895, p. 145). Stiles (1892) gave a fuller and illustrated account of the *Coccidium bigeminum* of the dog. He described the development of the oocyst with the production of two sporoblasts and two sporocysts, and the formation within each sporocyst of four sporozoites and a residual body. A figure of a section of the villus shows the presence of oocysts containing the sporoblasts or undeveloped sporocysts within the deep tissues of the villus. The size of the oocyst is given as 14 by 8 microns.

From the description of Railliet and Lucet, and Stiles, it is evident that they were dealing with the coccidium seen by Finck, Virchow and Rivolta in dogs and cats. These observations appear to be the last ones which have been made on the small *Isospora* of these animals.

Wasielewski (1904) gave a detailed account illustrated with excellent microphotographs of the oocysts of a coccidium, called by him *Diplospora bigemina*, which he had observed in cats that were used for experiments on amoebic dysentery by Jürgens. The oocysts which he observed varied in size, and he gives a series of



measurements in microns as follows:—22 by 19, 25 by 20, 25 by 22, 35 by 23, 35 by 25, 35 by 27, 38 by 32, 40 by 28. He describes the development of the oocyst in detail. The contents contract to form a sphere, which has a diameter of 18 to 25 microns according to the size of the cyst. Two daughter spheres are formed by division of the large sphere, and these vary in diameter from 16 to 18 microns in the larger oocysts and from 11 to 12 microns in the smaller ones. The daughter spheres become sporocysts, within each of which are developed four sporozoites 11 to 12 microns in length and a residual body 6 to 8 microns in diameter. The earlier stages of the parasite were found only in the epithelium of the small intestine and never in the submucosa, so that Wasielewski considered that the statements which had been made of a coccidium limited to the submucosa required some qualification. Schizonts in the epithelium and motile merozoites free in the lumen of the intestine were also seen.

This coccidium is clearly distinct from that studied by Finck, Virchow, Rivolta, Railliet and Lucet, and Stiles. From the size of the oocysts they appear to fall into two categories, as noted by Reichenow (1921), the one with oocysts measuring 22-25 by 19-22 microns and the other with oocysts measuring 35-40 by 23-32 microns. Those of the first category clearly correspond with the parasite described by Grassi (1879, 1882, 1883). Wasielewski also gave measurements of 18 by 25 microns for the oocysts and 11 by 15 microns for the sporocysts of a form seen by him in the dog. He regarded it as *Coccidium bigeminum*, but it corresponds exactly with Grassi's *Coccidium Rivolta*.

Basset (1909) without giving any description of the parasites, discusses the pathogenic effect of coccidia, which he calls *Diplospora bigemina*, in young dogs. He also records a round coccidium, 14 microns in diameter, as occurring in dogs and ferrets, but there is no evidence that these were actually coccidia, as no mention is made of any development.

Swellengrebel (1914) gave a complete account of the development of a coccidium of the cat under the name *Isospora bigemina*, which appears to be identical with the large form noted by Wasielewski. He described for the first time the process of schizogony in the epithelial cells of the small intestine, the evolution of the macrogametocytes and microgametocytes, and formation and



development of the oocysts. The measurements of the oocyst are given as 39 to 47 microns by 26 to 37 microns. The sporocysts vary in length from 21 to 24 microns, and in breadth from 18 to 19 microns. Within the sporocyst there are formed four sporozoites measuring 18 by 4 microns, and a large residual body. Swellengrebel clearly states that the appearances are absolutely unlike those figured by Stiles, but hesitates to establish a new species.

Weidman (1915) described a coccidium, which he called *Coccidium bigeminum*, in 'swift foxes' in the Western United States. The oocysts varied from 25 to 40 microns in length by 25 to 30 microns in breadth. The sporocysts measured 16 to 20 by 14 to 18 microns. Owing to the difference in dimensions from the form described by Railliet and Lucet, and Stiles, Weidman suggests the 'new varietal name "canivecolis".' He gives figures of the oocyst containing two sporocysts, with four sporozoites and a residual body. Mesnil (1916) states that Weidman regarded it as a variety *canivecolis* of *Isospora bigemina*.

Wenyon and O'Connor (1917) found an *Isospora* of the cat very common in Alexandria, and Dobell (1919) records a similar experience in England in the case of cats used for experiments on amoebic dysentery. In both these instances the oocysts were of the large type. This was also the writer's experience during experiments on cats conducted in London in 1912.

Hall (1917) discovered a coccidium in dogs in Detroit. On account of its large size, he thought it was different from *Isospora bigemina*, but later Hall and Wigdor (1918) concluded that it was a larger form of the same parasite, and wrote of it as *Diplospora bigemina*. The oocysts measured 36 to 40 microns in length by 28 to 32 microns in breadth. The sporocysts had a diameter of 10 to 20 microns, and the sporozoites measured 12 by 4 microns. Oocysts of these dimensions occurred in the majority of dogs, but in one animal a smaller strain was seen, the oocysts measuring 20 by 18 microns and the sporocysts 12 by 11 microns, with sporozoites 10 microns in length by 3 microns in breadth. They state that this distinction in the size was quite marked, and that it raises the question as to whether the small one should be regarded as a variety or species. They go on to say that it is possible that there are several species of *Diplospora* in the dog

characterised by considerable difference in size. The length of time required for the development of the oocyst of the larger form was two days when kept in 10 per cent. potassium bichromate solution. Under other conditions, which they state more nearly resemble those of nature, the time required may be two weeks or longer.

Reichenow (1921), referring to the *Isospora* of cats and dogs, expresses it as his opinion that Wasielewski was probably dealing with a mixed infection of two distinct coccidia in the cats he examined. He also states that the form he had observed in the dog in Germany differs from that in the cat, and resembles the one with smaller oocysts studied by Wasielewski. For the oocysts of the dog form he gives a length of 21 to 24 microns, and a breadth of 18 to 20 microns. The sporocysts, which are oval in outline, measure 14 to 16 microns by 9 to 10 microns. Nöller (1921), in a brief reference to the coccidium of dogs and cats, refers to the large and small form in cats and the one in dogs. He has been able to infect young dogs in series with the oocysts. No details of the dimensions are given. Marotel (1922) studied the *Isospora* of the cat. His measurements are as follows:—

Oocysts 45–48 × 34–36 microns.

Sporocysts 22–24 × 17–19 microns.

Sporozoites 18–20 × 4–5 microns, residual body in sporocyst 10–12 microns.

He proposes to call the coccidium *Isospora cati*. In order to facilitate the following discussion, the various dimensions in microns given by the above observers for the oocysts and sporocysts of the dog and cat parasites are arranged in tabular form:—

TABLE I.

						Oocyst	Sporocyst
Finck (cat)	...	...	...	...	...	...	8–10 × 7–9
Virchow (dog)	...	...	...	...	...	Like those described by Finck	...
Rivolta (dog and cat)	...	...	...	...	...	12–16 × 12	8–12 × 8
Grassi (cat)	...	...	...	...	...	27–30·8 × 22–24	14·3
Railliet and Lucet (dog)	...	...	...	...	...	12–15 × 7–9	...
— (cat)	...	...	...	...	...	8–10 × 7–9	...
— (pole cat)	...	...	...	...	...	8–12 × 6–8	...
Stiles (dog)	...	...	...	...	...	13·6–15·9 × 7·9–9·9	...
Wasielewski (cat)	...	...	...	...	...	35–40 × 23–32	16–18
— (cat)	...	...	...	...	...	22–25 × 19–22	10–12
— (dog)	...	...	...	...	...	18 × 25	11–15
Swellengrebel (cat)	...	...	...	...	...	39–47 × 26–37	21–24 × 18–19
Hall and Wigdor (dog)	...	...	...	...	...	36–40 × 28–32	10–20
— (dog)	...	...	...	...	...	20 × 18	12 × 11
Reichenow (dog)	...	...	...	...	...	21–24 × 18–20	14–16 × 9–10
Marotel (cat)	...	...	...	...	...	45–48 × 34–36	22–24 × 17–19

From the above table it will readily be seen that the oocysts described fall into three groups.

(1) There are the small forms described by Finck, Rivolta, Railliet and Lucet, and Stiles. Finck did not state the actual measurements of the oocysts, but from the size given for the sporocysts and the fact that in his description he says that two of these sometimes occur together enclosed by a common membrane, it is safe to assume that the oocyst would have dimensions similar to those described by Rivolta, Railliet and Lucet, and Stiles. The forms seen by Virchow are evidently similar, for he says they occur in the interior of the villi of dogs, are relatively small and regularly arranged in pairs enclosed by a thick, double contoured membrane. It is probable also that those described by Leuckart are of the same type.

(2) The second type has an oocyst of intermediate size. This was first seen by Grassi in the cat, later by Wasielewski in the cat and dog, by Hall and Wigdor in the dog, by Reichenow in the same animal, and possibly by Nöller in the cat and dog.

(3) The third type has an oocyst of much larger size. This was first definitely described by Wasielewski and Swellengrebel in the cat, and was seen by Wenyon and O'Connor, Dobell, Hall and Wigdor, and Marotel.

That these three types represent distinct species seems clear from the above records, and from observations to be recorded in this paper. In a recent study of English cats, the oocysts which occurred in the faeces were uniformly of large size, while those which were found in dogs' faeces were of the intermediate type. In one instance only was an infection of the cat with the small type seen. In this case the large form occurred also and it was clearly evident that the small one was limited to the deeper tissues of the villus, while the large one developed in the epithelium. Furthermore, development of many of the small oocysts was completed in the tissues of the villi, while those of the large form did not take place for some days after it had left the body. That the oocysts of the smallest form sometimes escape in the faeces in the undeveloped condition is demonstrated by an observation which has just been made by Mr. Leslie Sheather, of the Royal Veterinary College, with whom the writer has discussed his investigations on coccidiosis of dogs and

cats. By a process of concentration employed for the detection of worms' eggs in faeces, Mr. Leslie Sheather discovered that one dog was infected with the small form and another with that of intermediate size. The small oocysts measured about 12 microns in longest diameter, and like those of intermediate size were in the undeveloped condition. They proceeded to development when kept outside the body.

#### NOMENCLATURE

As regards the nomenclature of these parasites, there appears to be no great difficulty, though the name *Coccidium bigeminum* Stiles, 1891, has been employed indiscriminately for all three forms. Apart from Rivolta's name *Cytospermium villorum intestinalium canis* which he proposed in 1878, Grassi's name *Coccidium Rivolta* (1879) is the first one to be given to any one of these Coccidia. As pointed out above, Grassi was dealing with the oocysts of intermediate size in the cat, and, assuming that this form is the same as that of corresponding size from the dog, his name has priority. The name of this coccidium is, therefore, *Isospora rivolta* (Grassi, 1879). For the small form in the dog and cat the correct name is *Isospora bigemina* (Stiles, 1891). This leaves the large form in the dog and cat still unnamed, for the name *Isospora cati* suggested by Marotel (1922) cannot stand, as Railliet and Lucet (1891) employed the name *Coccidium bigeminum* var. *cati* for the small form in the cat, which if recognised as a distinct species from that in the dog, would become *Isospora cati*. For the large species the name *Isospora felis* is suggested. There are thus to be distinguished in dog and cat three species of *Isospora*:—

*Isospora bigemina* (Stiles, 1891).

*Isospora rivolta* (Grassi, 1879).

*Isospora felis* n. sp.

It is assumed that these different parasites are able to infect both dogs and cats, but it is possible that each animal has its own species. This can only be determined by more detailed observation and cross-infection experiments with clean animals. Railliet and Lucet (1891) have stated that the small forms in the cat, dog and pole cat are varieties of *Isospora bigemina*, while Weidman (1915) has made a similar suggestion for the large coccidium described by him



in the fox. His name was not properly proposed, as he merely says he advances a new varietal name 'canivecolis.' Mesnil (1916), in a summary of Weidman's paper, writes the specific name *canivecolis*, while Hall and Wigdor (1918) give it in full *C. bigeminum canivecolis*. This parasite, which is certainly not a variety of *Isospora bigemina*, may be identical with *Isospora felis*, but on the other hand it may be distinct. In the latter case the name *Isospora canivecolis* would be correct.

There exists also in dogs in England an intestinal *Eimeria* as recorded by Brown and Stammers (1922). For this parasite the name *Eimeria canis* is proposed.

Though Grassi (1879) proposed the name *Coccidium Rivolta* for the parasite he found in the cat, this name has been modified by several observers, in spite of the fact that Grassi repeated the name in his later papers (1882, 1883). Dobell (1919) in discussing this question, says that it is his view that Grassi's name should be changed by putting 'rivolta' in the genitive, in which case the name would be *Isospora rivoltae*. He thinks that a form such as 'rivoltai' is objectionable. There seems, however, to be no real reason why the name should be changed at all, and to keep it in the form proposed by Grassi is in accordance with Rules of Nomenclature. Both the changes discussed by Dobell have, however, been previously made. Thus in the English translation of Leuckart's work (1886) there appears a note on page 221 initialed by the author (R. L.) in which the name *Coccidium Rivoltae*, Grassi is used for the first time. Railliet (1895, p. 146) uses the name *Coccidium bigeminum* Stiles, 1891, for the small coccidium of the cat, dog and pole cat, and the name *Coccidium (?) Rivoltai* Grassi, 1881, for the form of intermediate size seen by Grassi. Neveu-Lemaire (1912) employs the name *Eimeria Rivoltai* Grassi, 1881, for the latter form, while Brumpt (1922), in the latest edition of his *Précis de Parasitologie*, uses the name *Isospora Rivoltai* Grassi, for all these parasites.

Several observers, including Wasielewski (1904), Martin (1909), Guiart (1910) and Hall and Wigdor (1918), place these parasites in the genus *Diplospora*, which, however, is generally recognised as a synonym of *Isospora*.

For convenience of reference, the following list of names



which have been employed for the *Isospora* of cats and dogs is appended:—

- Finck (1854). Corpuscules géminés.  
 Vulpian (1858). Corps oviformes.  
 Ercolani (1859). ? (Quoted by Rivolta and Perroncito.)  
 Virchow (1860). Psorospermien.  
 Leuckart (1860). Psorospermien.  
 Davaine (1860). Corpuscules géminés.  
 Leuckart (1863). Psorospermien.  
 Leuckart (1866). Psorospermien.  
 Eimer (1870). Psorospermien.  
 Zürn (1874). Psorospermien.  
 Rivolta (1874). Cellule oviforme.  
 Rivolta (1877). Cellule oviforme.  
 Rivolta (1877). Cellule oviforme.  
 Davaine (1877). Corpuscules géminés.  
 Rivolta (1878). *Cytospermium villorum intestinalium canis*.  
 Leuckart (1879). *Coccidium perforans*.  
 Grassi (1879). *Coccidium Rivolta*.  
 Grassi (1882). *Coccidium Rivolta*.  
 Bütschli (1882). *Coccidium Rivolta* Grassi.  
 Perroncito (1882).  
*Coccidium Rivolta*.  
*Cytospermium villorum intestinalium canis*.  
 Braun (1883). *Coccidium perforans*.  
 Grassi (1883). *Coccidium Rivolta*.  
 Balbiani (1884). *Coccidium perforans*.  
 Leuckart (1886). *Coccidium Rivoltae*, Grassi.  
 Railliet (1886). *Coccidium Rivolta* Grassi.  
 Pachinger (1887). Sporozoon.  
 Neumann (1888). *Coccidium perforans*.  
 Railliet and Lucet (1888). Corps oviformes.  
 Zürn (1889). *Coccidium oviforme* Leuck.  
 Blanchard (1889). *Coccidium Rivolta* Grassi, 1881.  
 Pfeiffer, L. (1890). Coccidien.  
 Railliet and Lucet (1890). Coccidies.  
 Stiles (1891). *Coccidium bigeminum*.  
 Pfeiffer, L. (1891). Coccidien.  
 Railliet and Lucet (1891). *Coccidium bigeminum* vars. *canis*, *cati*, *putori*.  
 Stiles (1892). *Coccidium bigeminum* Stiles, 1891.  
 Neumann (1892).  
*Coccidium bigeminum*.  
*Coccidium perforans*.  
*Coccidium Rivolta* Grassi.  
 Mosler and Peiper (1894). Coccidien.  
 Railliet (1895).  
*Coccidium bigeminum* Stiles, 1891.  
*Coccidium(?) Rivoltai* Grassi, 1881.  
 Braun (1895). *Coccidium bigeminum* Stiles 1891.  
 Moniez (1896). *Coccidium bigeminum* Stiles (1891).  
 Blanchard (1896). *Coccidium bigeminum* Wardell Stiles, 1891.  
 Labbé (1896). *Coccidium bigeminum* Stiles.  
 Wasielewski (1896).  
*Coccidium bigeminum* Stiles.  
*Coccidium spec. inc. Rivolta* Grassi.  
 Labbé (1899). *Coccidium bigeminum* Stiles.  
 Blanchard (1900). *Coccidium bigeminum* Wardell Stiles, 1891.  
 Neveu-Lemaire (1901). *Coccidium bigeminum* Wardell Stiles, 1891.  
 Doflein (1901). *Coccidium bigeminum* Stiles.  
 Perroncito (1901).  
*Coccidium bigeminum* Wardell Stiles, 1891.  
*Coccidium Rivolta*.  
 Neveu-Lemaire (1902). *Coccidium bigeminum* Wardell Stiles, 1891.  
 Neveu-Lemaire (1903). *Coccidium bigeminum* Wardell Stiles, 1891.  
 Braun (1903). *Coccidium bigeminum* Stiles, 1891.  
 Minchin (1903). *Coccidium bigeminum* vars. *canis*, *cati*, *putori* Railliet et Lucet.  
 Wasielewski (1904). *Diplospora bigemina*.  
 Neumann (1905).  
*Coccidium bigeminum*.  
*C. Rivoltae* (Grassi).  
 Guiart and Grimbert (1906). *Coccidium bigeminum* Stiles.  
 Lühe (1906). *Isospora bigemina* (Stiles).  
 Braun (1906). *Coccidium bigeminum* Stiles, 1891.

- Neveu-Lemaire (1906). *Coccidium bigeminum* Wardel Stiles, 1891.  
 Braun (1908). *Isospora bigemina* (Stiles) 1891.  
 Neveu-Lemaire (1908). *Coccidium bigeminum* Wardel Stiles, 1891.  
 Basset (1909). *Diplospora bigemina*.  
 Guiart and Grimbert (1908). *Coccidium bigeminum* Stiles.  
 Braun and Lühe (1909). *Isospora bigemina* (Stiles).  
 Martin (1909). *Diplospora bigemina* Stiles.  
 Doflein (1909). *Isospora bigemina* (Stiles).  
 Braun and Lühe (1910). *Isospora bigemina* (Stiles).  
 Brumpt (1910). *Coccidium bigeminum* Wardel Stiles, 1891.  
 Guiart (1910). *Diplospora bigemina*.  
 Doflein (1911). *Isospora bigemina* (Stiles).  
 Fiebiger (1912). *Isospora bigemina* Stiles.  
 Neveu-Lemaire (1912). *Eimeria Rivoltai* Grassi, 1881.  
 Jollos (1913). *Isospora bigemina*.  
 Brumpt (1913). *Coccidium bigeminum* (Wardel Stiles, 1891).  
 Swellengrebel (1914). *Isospora bigemina* (Stiles).  
 Braun and Seifert (1915). *Isospora bigemina* (Stiles) 1891.  
 Doflein (1916). *Isospora bigemina* (Stiles).  
 Fantham (1916). *Isospora bigemina*, Stiles, 1891.  
 Wenyon and O'Connor (1917). *Isospora* of cats.  
 Hall and Wigdor (1918). *Diplospora bigemina*.  
 Dobell (1919).  
*Isospora bigemina* Stiles.  
*Isospora rivoltai* Grassi (1879).  
 Reichenow (1921). *Isospora bigemina* (Stiles).  
 Dobell and O'Connor (1921). *Isospora rivoltai* Grassi.  
 Nöller (1921). *Isospora bigemina*.  
 Mayer (1922). Coccidien ?  
 Brumpt (1922). *Isospora Rivoltai* Grassi.  
 Marotel (1922). *Isospora cati*.

#### DESCRIPTION OF THE COCCIDIA OF CATS AND DOGS

During the course of certain observations on the faeces of dogs, the results of which have been published by Brown and Stammers (1922), it became evident that dogs were sometimes infected with a species of *Eimeria* in addition to the commonly recognised *Isospora*. The oocysts of the latter parasite agreed, as regards dimensions, with those given by Grassi (1879, 1882, 1883) for the *Isospora* of the cat, by Wasielewski (1904) for the *Isospora* of the dog and small form in the cat, and by Reichenow (1921) for one in the dog, and were constantly smaller than the oocysts of the *Isospora* of the cat which was under observation at the same time, so that it seems highly probable that the common *Isospora* of dogs and cats belong to two distinct species. The oocysts of the *Eimeria* of the dog varied considerably in size, some of them being as large as those of the large *Isospora* found in the cats, while others were smaller even than those of the *Isospora* seen in the dogs. They differed in appearance from the oocysts of the *Isospora* of the same animal and showed a

much greater range in size, but it was only after material had been kept till development of the oocyst had completed itself that it was definitely recognised as an *Eimeria*. It is possible that this *Eimeria* has been seen before and regarded as an *Isospora*, but of this there is no evidence.

In the case of the *Isospora* of the cat the oocysts examined by the writer have been constantly of large size, except in one instance when very much smaller ones were also present. It was found by examination of the small intestine of this cat that the large oocysts were derived from an *Isospora* (*Isospora felis*) which was undergoing development in the epithelium of the intestinal villi, while the very much smaller ones belonged to another *Isospora* (*Isospora bigemina*) which was parasitic only in the deeper tissues of the villi. Furthermore, the oocysts of the latter form completed their development in the tissues, whereas those of the large form in the epithelium were in the usual undeveloped condition. The faeces of this cat had been examined on several occasions in connection with experiments with *Entamoeba histolytica*, but the only oocysts noted in the faeces were the large undeveloped ones of *Isospora felis*. The oocysts of *Isospora bigemina* were not seen in the faeces, and if they had been present to any extent they could not have escaped recognition. They were first detected when a scraping of the wall of the small intestine was made with a view to finding amoebae which had been seen in this situation in another cat with amoebic dysentery. It seems clear that the oocysts of the small form do not escape into the faeces so regularly as do those of the large one which develops in the epithelium. There is no doubt that there were two distinct species of *Isospora* in this cat.

A detailed study of the development of *Isospora felis* and *Isospora bigemina* as they occurred in the tissues of cats was undertaken, but *Isospora rivolta* of the dog was only investigated in the oocyst stages which occurred in the faeces.

#### **ISOSPORA FELIS** n. sp.

The only complete account of the development of this common coccidium of the cat is that of Swellengrebel (1914), though Wasielewski (1904) had described the development of the oocyst and had seen other stages in the epithelium. In its main outlines

Swellengrebel's description is correct, but the growth of the microgametocyte was not fully traced. The supposed parthenogenesis of the macrogametocyte is capable of another interpretation, while the account of the changes undergone by the nuclei requires revision. It seems, therefore, desirable to redescribe the life-history as it has been studied in sections of the epithelium of the small intestine of cats.

*Schizont.* The smallest forms which can be found in the epithelial cells are only 5 microns in length (Plate IX, fig. 1). These are curved and somewhat sickle-shaped bodies which are pointed anteriorly and rounded posteriorly. They lie in vacuoles in the cells, and are attached to the cytoplasm of the cell by the pointed extremity. The nucleus is spherical and has a definite membrane. Within the nucleus is a body which, in staining reactions, does not appear to be rich in chromatin. It is usually applied to the nuclear membrane. In addition the nucleus contains a granular material which is probably chromatic in nature. Whether the large body should be regarded as a karyosome depends on the definition of this term. It does not stain intensely with Mayer's haemalum, has the appearance of plastin material rather than chromatin, and in this respect resembles a nucleolus rather than a karyosome. Growth of the parasite takes place till it has a length of about 10 microns and a diameter at its thickest part of about 5 microns (Pl. IX, figs. 2 and 3). Though plumper than the youngest forms, it still retains its elongate gregariniform character. While still in this condition nuclear division commences (Pl. IX, fig. 4) by division of the karyosome. The daughter karyosomes take up positions at the end of the now elongated nuclear membrane as two polar caps while a definite equatorial plate of small chromosomes is formed (Pl. IX, fig. 5). Two daughter plates are formed and the first nuclear division is completed by division of the membrane (Pl. IX, figs. 6-8). The two daughter nuclei have the same structure as that of the original nucleus. The second nuclear division takes place in a similar manner, as does also the third, though the karyosomes as a rule become smaller with each division (Pl. IX, figs. 9-12). When eight nuclei are present, the parasite has become more definitely ovoid in shape, and eight merozoites are formed by a budding process which leaves a definite residual body. Eight appears to



be the usual number for the merozoites, for the vast majority of schizonts which have been seen are of this type. The size of the merozoites, however, varies considerably even when only eight are present (Pl. IX, figs. 14 and 15). It seems possible that the small forms are destined to develop again into schizonts and the larger ones into gametocytes, but no definite proof of this could be obtained. Occasionally a smaller number of merozoites appeared to be formed (Pl. IX, fig. 16), but in such cases it is possible that the appearance was due to multiple infection of a cell by merozoites after schizogony had occurred, or to the fact that all the merozoites resulting from schizogony had not escaped from the cell. In several instances the occurrence of two merozoites in a single vacuole was undoubtedly due to two merozoites having invaded the same cell simultaneously. On the other hand, a large number of merozoites is sometimes formed, as pointed out by Swellengrebel (Pl. IX, figs. 17 and 18). In several instances as many as sixteen occurred, while a larger number was once seen. These forms, however, occurred rarely in the material examined, and, as stated above, the great majority of schizonts produced only eight merozoites.

It should be pointed out that the schizonts tend to stain very deeply, even with very dilute Mayer's haemalum, which proved to be the most satisfactory stain for these forms, so that unless thin sections are examined there may be considerable difficulty in making out the details of the nuclear divisions.

During growth the schizont is closely applied to the nucleus of the host cell, which becomes definitely altered in character.

*Microgametocyte.* The microgametocyte possibly commences as one of the larger merozoites (Pl. IX, fig. 19). Like the schizont, it retains, for a considerable period of its growth, its gregariniform character. When it has a length of about 12 microns (Pl. IX, fig. 20) the first nuclear division takes place. This is very similar in character to that of the schizont. The karyosome is present and divides in the same manner by dumb-bell constriction, while there is evidence that chromosomes are also formed (Pl. IX, figs. 20-22). Repeated nuclear divisions of the same type take place while the microgametocyte increases steadily in size. It finally loses its gregariniform character and becomes irregular in shape till it has a length of about 20 microns (Pl. IX, figs. 23-28). The increase in



bulk up to this stage has been relatively enormous. The details of the nuclear divisions are difficult to follow owing to the marked affinity the cytoplasm has for stains. This obscures details to such an extent that it is very difficult to detect the arrangement of the chromatin during the divisions of the nucleus.

After this a change takes place. The cytoplasm ceases to stain intensely, the chromatin material in the nucleus becomes much more definite and the karyosome decreases in size. Nuclear divisions continue, and these are definitely mitotic in character (Pl. X, figs. 1-3). The chromosome number has not been counted with accuracy, but it appears to be somewhere within the limits of 8 and 12. It appears that the nuclear membrane persists throughout nuclear division. The cytoplasm becomes fissured in various ways and loses still more its affinity for stains. Finally, when nuclear division is complete, the microgametocyte contains a large number of nuclei which have definite nuclear membranes within which are irregular masses of chromatin (Pl. X, fig. 4). The karyosome, which had decreased in size during the later divisions, is no longer clearly visible, but it seems probable that it is still present, for in the later divisions of the nucleus it is often possible to detect a small granule at each end of the mitotic figure. These two granules may be united by a fibre, so that the appearance of a minute karyosome dividing by elongation and constriction is produced. The nuclei then shrink, and become compact, deeply staining masses of chromatin (Pl. X, fig. 5).

Formation of microgametes commences by the outgrowth from the nucleus of a short process (Pl. X, fig. 6). The whole nucleus then elongates (Pl. X, fig. 7), and it seems probable that the short process represents the anterior end of the microgamete. The short curved masses then become more elongate, and fine tapering microgametes about 5 microns in length are formed (Pl. XI, fig. 1). The cytoplasm of the microgametocyte either collects into a single large residual body on the surface of which the microgametes lie, or it breaks up into several separate masses. A certain number of deeply staining granules remain in the residual body. The individual microgamete is pointed anteriorly and fine and tapering posteriorly. Sometimes there appeared to be a deeply staining granule near the anterior end of the microgamete. It is possible

that this granule functions as a blepharoblast from which the two flagella which Swellengrebel demonstrated arise. It seems probable that this granule is the karyosome or, as some would term it, the centriole which could be detected during the later divisions of the nuclei of the microgametocyte. When development of the microgametocyte is completed it may have a length of nearly 50 microns and measure over 30 microns in the two other diameters, so that it appears in many sections of a series. Well over two thousand microgametes may be formed by each microgametocyte. Swellengrebel was unable to trace the complete development of the microgametocyte, but it appears from his figures that some of the forms which he regarded as developmental stages of schizonts are really microgametocytes.

*Macrogametocyte.* It is assumed that the macrogametocyte commences as one of the larger merozoites (Pl. XI, fig. 2). At this early stage it has been impossible to differentiate between the young stages of either the microgametocytes, macrogametocytes or schizonts. The macrogametocyte can, however, be recognised at later stages owing to the fact that it has increased in size without nuclear division. It retains its gregariniform character, and is attached to the surface of the vacuole in the cell by its pointed extremity. The attachment is frequently on the nucleus, which in some cases is drawn into the vacuole (Pl. XI, figs. 3 and 4). On several occasions what appears to be a definite organ of attachment was seen (Pl. XI, fig. 5). Sometimes there is an appearance of a terminal sucker which has drawn into it a small pedicle of the cytoplasm of the cell. Even when the macrogametocyte reaches a large size the gregariniform shape is retained, so that the parasite may become doubled to accommodate itself to the space at its disposal (Pl. XI, figs. 6-8). During the stages of growth represented by Pl. XI, figs. 2-8 the cytoplasm stains deeply, with a tendency towards the accumulation of more intensely staining material round the nucleus in the later stages. The nucleus has increased considerably in size and possesses a large karyosome which has little affinity for stains. A change now takes place in the staining reactions. The deeply staining material round the nucleus increases in amount and there appears in the cytoplasm a number of deeply staining irregular bodies, while the cytoplasm itself becomes filled with vacuoles containing a clear refractile substance

(Pl. XI, fig. 9). The cytoplasm generally has less affinity for stains than it had previously, and it seems as if the substance which caused the cytoplasm to stain deeply in the earlier stages has now become aggregated in the irregular masses. The latter eventually disappear, leaving a clear cytoplasm filled with refractile globules (Pl. XI, figs. 10 and 11; Pl. XII, fig. 1). Finally the oocyst is secreted round the macrogametocyte. It does not become thick or resistant till it leaves the cell, for in fixed tissues the oocysts within the cells are permeable to fixatives and show no signs of the shrinkage and lack of proper fixation which is characteristic of those which are free in the lumen of the intestine.

During the growth of the macrogametocyte it is frequently noted that a granular substance accumulates in the vacuole between the parasite and the wall of the vacuole. This material, which often stains brilliantly with eosin, causes indentations in the macrogametocyte in various places (Pl. XI, fig. 11). Similar accumulations sometimes occur in the case of the microgametocytes (Pl. X, fig. 1).

Swellengrebel described a process of parthenogenesis of the macrogametocyte. Nothing comparable with this has been seen during the present investigations, and, judging from his figures, it seems that the stages he figures, in which definite nuclei are not present, are drawn from sections of macrogametocytes which did not include the nucleus but showed the deeply staining material which occurs around it (Text-fig. 2, p. 259). The large macrogametocytes naturally occur in several sections of a series, and the nucleus may only be found in one of these. In the sections on either side of this one the macrogametocytes will have the appearance of the forms figured by Swellengrebel as illustrating his process of parthenogenesis.

The foregoing description of the development of *Isospora felis* in the intestinal epithelium of the cat is of interest from several points of view. In the first place it is of importance to note that the parasite is limited to the epithelial cells. In no case has it been seen in the sub-epithelial tissues. The infection, moreover, appears to be confined almost entirely to the epithelium near the distal ends of the villi, there being little tendency for it to spread towards their bases.

During the growth of the young forms of the schizont and

gametocyte the parasite retains its gregariniform character to a relatively late stage. In this respect *Isospora felis* differs from many coccidia, which quickly assume the spherical form when growth commences. The fixation of the growing forms to the surfaces of the vacuoles by the pointed end and the development of what appears to be a definite organ of fixation still further increases the resemblance to certain gregarines, such as those of the genus *Lankesteria*.

The development of the microgametocyte merits special attention from the point of view of the behaviour of its nucleus. Schaudinn (1900), in his description of *Eimeria schubergi*, stated that the nucleus of the microgametocyte broke up into a chromidium, the granules of which collected in the form of a number of nuclei on the surface. A similar process was described by him (1902) for *Cyclospora caryolytica*, and again by Schaudinn and Siedlecki (1897) in the case of *Eimeria lacazei*. The majority of observers who have described the development of the microgametocytes of coccidia have followed Schaudinn in supposing that the numerous nuclei are formed from the chromidium into which the single nucleus breaks up. It was shown by Schellack (1912, 1913) and by Schellack and Reichenow (1913, 1915) for a number of coccidia, including the forms with which Schaudinn himself worked, that the latter's statements were incorrect, and that the nuclei of the mature microgametocyte resulted from repeated nuclear divisions from the original nucleus. A similar process had been described by Wasielewski (1904) for *Isospora lacazei* of birds, by Stevenson (1911) in the case of the *Eimeria* of the goat, by Léger and Duboscq (1910) for *Selenococcidium intermedium*, and by Siedlecki (1899) for *Adelea ovata*. It is very doubtful, therefore, if the microgamete nuclei are ever formed from chromidium, as Schaudinn maintained. It seems far more probable that in all coccidia they result from repeated nuclear divisions, as described above for *Isospora felis*.

The structure which has been called the karyosome is present in all the stages of schizogony and in the merozoites. It occurs during the early nuclear division stages of the microgametocyte, but in the later stages is represented by a minute granule. Whether this is to be regarded as a centrosome or centriole is a difficult question to decide. It certainly occupies the position in mitotic division that



a centrosome would occupy, and furthermore, it is probably this granule which occurs at the anterior end of the microgamete, and from it the flagella may originate. The karyosome is constantly present during the growth of the macrogametocyte, though it usually becomes smaller towards its maturity. Whether it disappears before fertilisation takes place has not been definitely determined, but it is certainly present during the nuclear division of the zygote and sporoblast. There was no indication that the karyosome was discharged from the nucleus prior to fertilisation. Though the latter process was not actually observed in stained preparations, in a few cases the nucleus of the fully grown macrogametocyte was elongated. It seems probable that this was an elongation preparatory to fertilisation, and if so it is worthy of note that the karyosome was still present in the nucleus.

*Oocyst.* As regards the oocysts themselves (Pl. XII, figs. 12-15) the measurements of a large number showed that they vary in length from 39 to 48 microns and in breadth from 26 to 37 microns, the majority measuring about 45 by 33 microns. These figures are practically identical with those given by Swellengrebel. Wasielewski, however, saw smaller forms in the cat, his measurements being 22 to 40 by 19 to 28. It seems possible that cats may be infected with both *Isospora felis* and *Isospora rivolta*, in which case Wasielewski's figures would cover a mixed infection with these two forms. Grassi appears to have been dealing with a pure infection of *Isospora rivolta* in the cat.

The development of the living oocyst of *Isospora felis* has been followed by Wasielewski, Swellengrebel and others, and there is little to add to their descriptions. Owing to the impermeable nature of the oocyst wall, it is difficult to obtain satisfactorily fixed preparations of the nuclei during its development. A certain number of preparations was, however, obtained in the following manner. Small quantities of the material containing oocysts in various stages of development were crushed between a slide and cover-glass in order to rupture the cysts, and films fixed in Schaudinn's fluid and stained with iron haematoxylin were made in the usual manner. There was thus obtained a number of stained preparations of the different stages.

The nucleus of the zygote (Pl. XII, fig. 2) has very much the



same appearance as that of the macrogametocytes in the tissues. A karyosome is still present, though it appears to be smaller. The same type of nucleus occurs in other stages, including those of the sporoblasts (Pl. XII, figs. 3-7), but in these the karyosome has increased relatively in size. A few nuclear divisions were seen, but these were not sufficiently numerous for many details to be made out. The stages which were seen resembled those which occur in the early stages of development of the microgametocyte, except that the karyosomes are smaller. The nuclei in various stages of development of the oocyst are depicted in Pl. XII, figs. 2-10.

The zygote nucleus (Pl. XII, fig. 2) is a spherical body consisting of a definite membrane, within which a number of fine granules and one larger mass—the karyosome—occur. Whether the karyosome is present in the earliest stage of the zygote nucleus could not be determined, as stained preparations of the fertilisation process were not seen. Satisfactory pictures of the first nuclear division were not observed, so that no statement can be made regarding a possible reduction in the number of the chromosomes. The two nuclei of the binucleate stage are shown at Pl. XII, fig. 3. Both nuclei are decolorized, and the small granule at the centre of the karyosome is well seen. The single nuclei of the two sporoblasts have the same structure. The first division in the sporoblast is shown at Pl. XII, fig. 4. The daughter karyosomes occupy the poles of the spindle, while daughter plates of chromosomes are also present. The nuclei of the binucleate stage of the sporoblast are shown at Pl. XII, fig. 5, and here again the nuclei are of the same type. The second nuclear division in the sporoblast shows two spindles with the karyosomes at the poles of the spindle, and definite equatorial plates (Pl. XII, fig. 6). The resulting four nuclei, with somewhat deeply stained karyosomes, are shown at Pl. XII, fig. 7.

Good preparations of sporozoites were fairly numerous. These measured from 10 to 15 microns in length (Pl. XII, figs. 8-11), being smaller after fixation than in the living condition. In some a large vacuole occurs near one end. This is evidently the position of the refractile body often seen in the living sporozoites (Pl. XII, fig. 15). The nucleus was spherical and contained a relatively large karyosome. In specimens from which the stain had been sufficiently extracted (Pl. XII, fig. 8) the karyosome was pale, and at its centre

was a small deeply staining granule. It thus appears that the karyosome is present in all stages of the nuclei during sporogony, though varying considerably in size.

The sporozoites appear to be budded off in pairs from the ends of the sporoblast. Two buds appear at each end, and these grow into elongate finger-like processes into which the nuclei enter. During their growth they turn over the surface of the residual body and lie between it and the wall of the sporocyst.

An important point to note is that the oocyst commences to form as a thin membrane while the macrogametocyte is still within the epithelium, but it does not become a resistant structure till the macrogametocyte has left the cell for the lumen of the intestine. In no case was there any indication that the further development of the contents took place, either in the cells or in the lumen of the intestine. Retraction of the zygote and division of the latter into two sporoblasts, which are the first steps in the development after the oocysts leave the body, were never noted in the case of oocysts within the epithelium or in the lumen of the intestine. It follows, therefore, that whenever observers have described the occurrence of paired bodies in the intestine wall they cannot have been referring to the oocysts of *Isospora felis*.

#### **ISOSPORA BIGEMINA (STILES, 1901)**

This coccidium was discovered in one cat which had been employed for experiments with *Entamoeba histolytica*. The cat died during the night, but at the autopsy next morning it was still warm and perfectly fresh and the amoebae active and in healthy condition. The cat had evidently been dead only a short time. It is important to note this fact, for many of the oocysts of *Isospora bigemina* which occurred in the submucosa were fully developed. It seems hardly possible that they could have completed their development in the short time following the death of the cat. This is all the more probable in view of the fact that oocysts of *Isospora felis* which were also present in the intestine were quite unchanged. It can safely be assumed, therefore, that the appearance of *Isospora felis* in the tissues and in the intestine were those which occurred during life. This accords with the descriptions which have been given by Finck, Virchow, Leuckart, Railliet and Lucet, and Stiles.

The sporocysts of this coccidium were first seen in scrapings of the wall of the small intestine after the death of the animal. It was at first thought that they were fully developed sporocysts of *Isospora felis*, but their small size was against this view. Further examination showed that they really occurred in pairs enclosed in an oocyst which was easily ruptured between the slide and cover-glass. The sporocysts had fairly thick, double-contoured walls, and contained four sporozoites and usually a residual body. The oocyst wall enclosing them was of a more delicate nature, and was closely wrapped round the two sporocysts. There was no indication of a micropyle in the oocyst.

Sections of the small intestine showed that the parasite did not occur in the epithelium, but was limited entirely to the sub-epithelial tissues of the villi, especially near their distal ends, some of which were swollen and packed with oocysts in various stages of development. The epithelium contained *Isospora felis*, which on account of its large size contrasted very markedly with the much smaller form in the tissues. Text-figure 2 is from a drawing of a transverse section of a villus, and shows two macrogametocytes, one with a nucleus and the other with the central granular mass to the side of the nucleus, a fully developed microgametocyte with microgametes, and a young micro- or macrogametocyte of *Isospora felis* in the epithelium, and six fully developed oocysts of *Isospora bigemina* in the sub-epithelial tissues.

The earliest stages of *Isospora bigemina* are seen as minute spherical bodies enclosed in vacuoles in the cytoplasm of mononuclear cells (Pl. XIII, fig. 1). Whether these are endothelial cells or not has not been determined. No endothelial cells which were evidently on the walls of blood vessels were found infected. These young forms are barely 2 microns in diameter. They grow into schizonts which are 5 to 6 microns in diameter, and produce about twelve merozoites. Owing to their small size, it is exceedingly difficult to follow the development in the sections (Pl. XIII, figs. 2-4). The microgametocytes have not been definitely identified, though several structures have been seen which are possibly of this nature. One of these has been drawn (Pl. XIII, fig. 5), and it would seem not improbable that the minute curved bodies are microgametes surrounding a residual mass of cytoplasm. The macrogametocyte

develops into an ovoid body 10 to 12 microns in length (Pl. XIII, figs. 6 and 7). It becomes enclosed in an oocyst (Plate XIII, fig. 8), within which it divides into two sporoblasts, which in their turn form sporocysts the walls of which are thicker than that of the oocyst. Within each sporocyst four sporozoites are produced (Pl. XIII, figs. 9-11). In many sporocysts it has been impossible to recognise

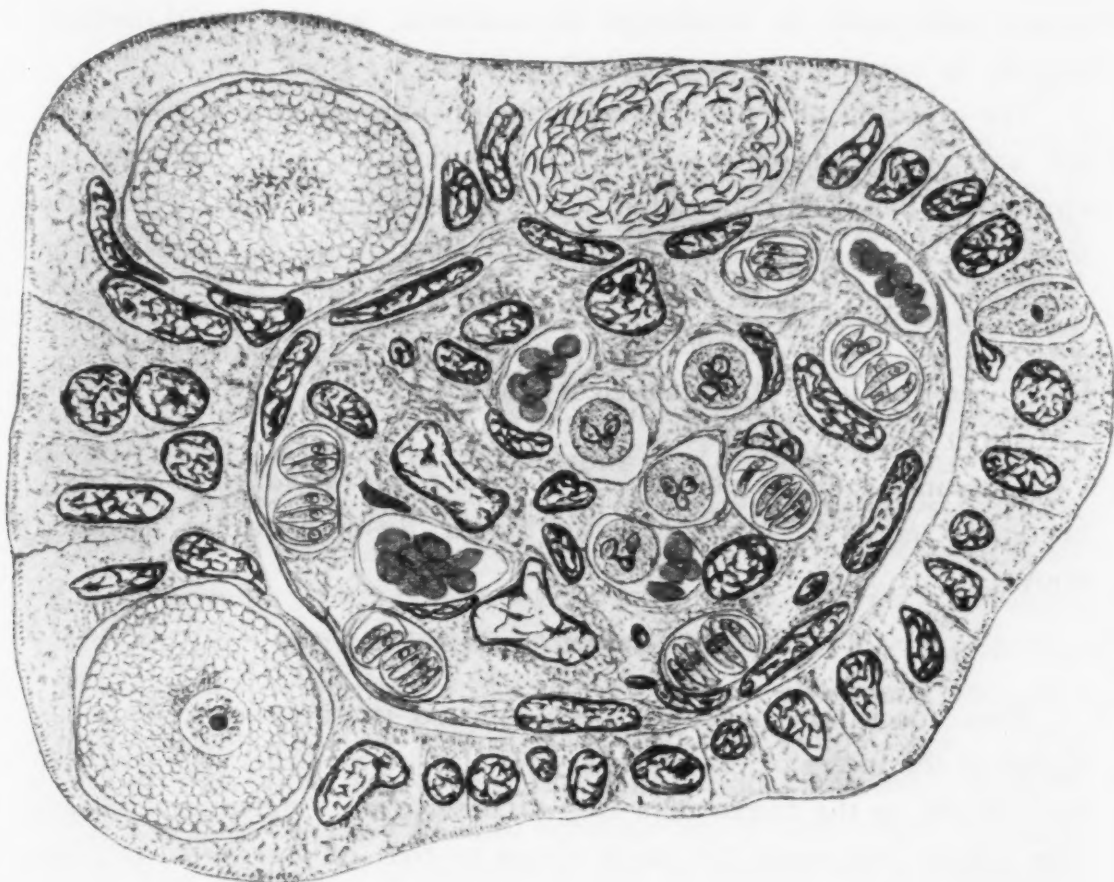


FIG. 2. Section of a villus of the cat showing *Isospora felis* in the epithelium and *Isospora bigemina* in the deeper tissues. In the epithelium are seen two macrogametocytes, one cut through the nucleus and one cut to the side of the nucleus: one microgametocyte which has given rise to numerous microgametes and a residual body, and one young form which may be a young macrogametocyte. In the interior of the villus are seen six mature oocysts of *Isospora bigemina*.  $\times 1500$ .

a residual body, but such a structure is definitely present in some cases. It appears that it breaks up and disintegrates after the sporozoites have been formed.

As regards the fate of the fully formed oocysts, there is no definite information to offer, except that they were not detected during the examination of the faeces made before death. In the sections of the intestine the epithelium was in many cases absent, so that escape



of the sporocysts would be an easy matter if such a change occurred in life. The heavily infected villi were considerably altered in appearance. They were swollen, and an excess of cells was present. It seems probable that such altered villi would break down during life and liberate the oocysts. These would not appear in the faeces regularly, as in the case of *Isospora felis* which develops in the epithelium, but would occur at intervals, whenever a villus broke down sufficiently to discharge its contents, which would include oocysts in various stages of development.

The question of a possible relationship between this parasite and the very much larger *Isospora felis* which develops only in the epithelium has been considered. It might be urged that if *Isospora felis* developed in the sub-epithelial tissues it might take on the character of the smaller form. The latter, however, has only been seen in one animal, while many infected with *Isospora felis* alone have been studied. It seems clear, therefore, that the small form is a distinct species.

The undeveloped oocysts of *Isospora bigemina* have recently been detected in the faeces of a dog by Mr. Leslie Sheather, as noted above.

#### *ISOSPORA RIVOLTA* (GRASSI, 1879)

This coccidium has only been studied in the oocyst stage as found in the faeces of three dogs. Mr. Leslie Sheather has also seen the oocysts in the faeces of a dog at the Royal Veterinary College. The oocyst has much the same shape as that of *Isospora felis*, but is smaller. The measurements obtained from the three dogs agree very closely with those given by Grassi (1879, 1882, 1883) for the form in the cat, and Reichenow (1921) for the one in the dog. The dimensions given by Wasielewski (1904) for the oocysts seen by him in the dog are very much the same, as also the smaller series found by him in the cat. Hall and Wigdor (1918) evidently met with this parasite in one dog. The reference made by Nöller (1921) to a large and small form in the cat may refer to *Isospora felis* and *Isospora rivolta*.

Four stages of development of the oocyst are shown at Pl. XIII, figs. 12-15. As seen in English dogs, they vary in length from 20 to 24 microns and in breadth from 15 to 20. Large oocysts



like those of the cat are never seen, though, as pointed out above, when the larger oocysts of *Eimeria canis* were present, it was at first thought that these belonged to *Isospora felis*. The difference in size between the oocysts of *Isospora felis* and *Isospora rivolta* was so constant that there can be little doubt that two species are represented, as Reichenow (1921) has suggested.

**EIMERIA CANIS** n. sp.

The oocysts of this coccidium were seen in three dogs, as recorded by Brown and Stammers (1922). In two of them the infection was a small one, while in the other it was fairly heavy. The remarkable feature of the oocyst is its great range in size. In this respect it resembles *Eimeria deblickei* of the pig, the oocysts of which were described by Cauchemez (1921). Another feature which is of interest in the case of *Eimeria canis* is that the sporocysts show the same proportional variation in dimensions as do the oocysts. It is evidently incorrect to suppose that in coccidia the sporocysts remain fairly constant in size in spite of variations in the dimensions of the oocysts. The oocyst of *Eimeria canis* varies in length from 18 to 45 microns, and in breadth from 11 to 28 microns. The general shape of the oocyst will be appreciated from the figures (Pl. XIII, figs. 16-19, and Pl. XIV, figs. 1-8). The cyst wall constantly had a peculiar pink colour, and what seemed to be the true oocyst wall was enclosed by a somewhat irregular thick membrane which gradually peeled off during the development outside the body. When this membrane had separated, the colour of the cyst was still the same, though much paler. The course of the development is illustrated in the drawings. It will be noted that a definite micropyle could be detected in some oocysts (Pl. XIII, figs. 16 and 18, and Pl. XIV, fig. 1) and that the enclosed cytoplasm was sometimes attached to it by a strand (Pl. XIII, fig. 16). An inner membrane indicated by radiating lines could also be detected in some of the oocysts (Pl. XIII, figs. 18 and 19, and Pl. XIV, figs. 1 and 2). During the formation of the sporoblasts there was a striking resemblance to *Eimeria stiedae* of the rabbit, as described by Metzner (1903). Pyramidal elevations with clear hyaline apices were formed. The sporocysts had the characters shown in the

drawings (Pl. XIV, figs. 5-8). It will be noted that at the narrower end there is a definite elevation or knob. In many respects the oocysts resemble those of the coccidium of the rabbit. Since the paper by Lucet (1913) appeared, it has been assumed that there are two coccidia in the rabbit, the one, *Eimeria stiedae*, with larger oocysts than the other, *Eimeria perforans*, as first clearly stated by Leuckart (1879). The former, according to Reichenow (1921), who agrees with this view, occurs in both the liver and intestine. In some cases the liver alone is infected, in others only the intestine, while in other animals both are found to harbour the coccidium. The other form, *Eimeria perforans*, is apparently limited to the intestine, though information on this point is not very definite. There seems, however, no reason to suppose that the coccidium of the dog represents two species, though in many respects it corresponds with a mixed infection of two forms in the rabbit. The great variation in size of the oocysts of *Eimeria canis* raises the question as to whether there are actually two coccidia in the rabbit or only one.

It does not seem possible to identify the form in the dog with the common rabbit coccidium, though Bruce (1919) has described a coccidium of the rabbit in America the oocysts of which resemble those of *Eimeria canis* in the presence of the layer of material covering the wall, in its pinkish orange colour and the marked range in size. Bruce was inclined to regard this parasite as a new species, or a variety of the common rabbit coccidium. It certainly resembles *Eimeria canis* more than any other recorded coccidium.

It should be mentioned, however, that Guillebeau (1916) has described a still smaller coccidium, which he says occurs in the liver cells of dogs. He identified it with *Eimeria stiedae*, though the oocysts measured only 12 by 7 microns. As pointed out by Reichenow (1921), the situation of the parasite in the liver cells is a most unusual one for coccidia. The figures given by Guillebeau do not assist in arriving at a conclusion as to the nature of the organism. Chierici (1908), quoted by Martin (1909), recorded a coccidium which he found in the bile of a cat. The oocysts had a thick, double-contoured wall, were oval in shape, and measured 26 to 30 microns in length by 17 to 20 microns in breadth. Development with the formation of four sporocysts, each with two sporozoites,

occurred. It is evidently a coccidium of the genus *Eimeria*, but whether it is identical with *Eimeria canis* cannot be determined.

Virchow (1865, p. 356) records his discovery in the gall-bladder and bile ducts of one dog of numerous egg-shaped psorosperms with thick, double-contoured shells. No further description is given, so that it is not possible to form an opinion as to whether these were oocysts of coccidia or eggs of a trematode. Another reference to similar structures is by Rivolta (1878), who gives the name *Cytospermium hepatis canis familiaris* to certain oval bodies which Perroncito had found in the bile ducts of the dog and which he had called *cellule oviforme del fegato del cane*. Perroncito (1882, p. 98) refers to what are evidently these bodies as '*Cytospermio del fegato del cane*.' He gives also the name '*Cellule oviforme del fegato del cane, Perroncito*.' They are described as measuring 48 to 52 microns in length by 21 to 32 microns in breadth. There is a capsule 2 microns in thickness, and at one pole an operculum. The contents divide into two to eight masses. There is little doubt that these bodies are eggs of a trematode. It appears that the first reference was made by Perroncito (1876), but this paper has not been consulted.

#### ISOSPORA OF MAN

The facts which have been explained above have a direct bearing on the status of the *Isospora* which has been recorded from human beings. It will be necessary to review the history of the discovery of the parasite. The first record of the occurrence of such a coccidium is that of Virchow (1860, p. 527), who mentions a case which was brought to his notice by Kjellberg. He found at post-mortem Psorosperms in the villi, which agreed entirely with those that he (Virchow) had seen in dogs ('*welche ganz mit denen übereinstimmen, die ich beim Hunde gesehen habe*'). The Psorosperms occurred in the interior of the villi, and especially towards their ends ('*in dem Innern und zwar gegen die Spitze der Darmzotten*'). Of the form seen by him in the dog, he says that in the interior of the villi he saw numerous Psorosperms of relatively small size regularly arranged in pairs with a double-contoured membrane ('*Indess habe ich neulich erwähnt (S. 342), dass ich in einem Hunde im Innern der Darmzotten sehr häufig Psorospermien*

antraf; es waren relativ kleine, regelmässig zu zweien aneinander gesetzte mit starker, doppeltcontourirter Membran versehene Körper'). He goes on to state that they must have been like the forms seen by Finck in the cat. From Virchow's statements, the only conclusion justifiable is that he saw in man a small coccidium like *Isospora bigemina* of the cat and dog.

The next reference is that by Eimer (1870), but this is much less satisfactory than that of Virchow. Eimer says that he saw Psorosperms in two men who were examined post-mortem in Berlin. The intestinal canal was described as being filled and the epithelium completely infiltrated with Psorosperms. He says they were like those seen by him in mice and other animals. In both the human cases the epithelium of the greater part of the intestine is described as having been devoured by the Psorosperms, as occurs in infected mice. The contents of the Psorosperms were finely granular. Eimer furthermore states that he observed all stages of the division of the contents, but gives no clear account of the process. From these meagre details it appears impossible to identify the Psorosperms seen by Eimer. Whether they were coccidia at all is far from clear. They evidently did not show the same arrangement in pairs noted by Virchow, for such a striking appearance would hardly have escaped his notice. The only points in favour of the view that they were coccidia are the statements that they occurred in the epithelium, and that they resembled undoubted coccidia of the mouse and other animals. As coccidia belonging to both the genera *Isospora* and *Eimeria* occur in man, it is fruitless to speculate as to which genus the form seen by him belongs.

Rivolta (1873) describes certain corpuscles he found in the faeces of man, but there is no evidence whatever that these were oocysts of coccidia. Similarly, the bodies seen by Grassi (1879), and which he regarded as coccidia, were probably cysts of *Giardia*. Rivolta (1879) proposed the name *Cytospermium hominis* for the psorosperms found in man by Eimer. The name is given explicitly to Eimer's psorosperms, and Rivolta makes no mention of the bodies originally described by him in 1873. Thus Rivolta's name *Cytospermium hominis* was given to certain bodies seen by Eimer which may or may not be coccidia, and even if they were coccidia are quite unidentifiable.



Railliet and Lucet (1890) described the small coccidium of the villi of dogs. They recognise in these the form named *Cytospermium villorum intestinalium canis* by Rivolta (1878). They correctly followed the development with the production of two sporoblasts, each of which gave rise to a sporocyst containing four sporozoites. The oocysts measured 12 to 15 microns by 7 to 9 microns. They state that they had seen coccidia in the faeces of a woman and her child who were suffering from chronic diarrhoea. The coccidia were regularly ovoid, and some of them contained granular protoplasm, including a number of refringent globules. Others contained a large granular mass without globules. The average size was 15 by 10 microns. They recognise, however, that they differed in certain respects from the forms seen in the dog.

In a later paper, Railliet and Lucet (1891) accept the name *Coccidium bigeminum* given by Stiles (1891) to the small coccidium of the dog. As pointed out above, they recognised three varieties of this organism, *Coccidium bigeminum* vars. *canis*, *cati* and *putori* in the dog, cat and pole cat, respectively. They say that a fourth variety probably also exists, namely, *Coccidium bigeminum* var. *hominis*, the form which was seen by Kjellberg and described by Virchow (1860). They make no mention of the bodies described by themselves in 1890. Railliet (1895), however, ascribes to the species *Coccidium bigeminum* the often quoted parasite discovered by Kjellberg. As regards the bodies seen by Railliet and Lucet (1900) in two human cases, Railliet groups them with those described from man by Grassi and Rivolta as doubtful forms about which it is not possible to express an opinion. He states, however, that the size of those recorded by Railliet and Lucet (15 by 10 microns) brings them into relation with *C. bigeminum*. Six pages further on in his book, Railliet again asserts that the parasite discovered by Kjellberg must without doubt be placed in this species (*C. bigeminum*), as it was situated in the interior and towards the tips of the villi, and resembled the form seen by Virchow in the dog. It is thus evident that Railliet and Lucet, in employing the name *Coccidium bigeminum* var. *hominis*, were naming not the form seen by themselves, but Kjellberg's parasite recorded by Virchow (1860).

From what has been said above, it will be apparent that in only one of the records, namely that of Virchow, is it possible to make



an accurate deduction that a coccidium was being dealt with. Rivolta's name *Cytospermium hominis* refers to Eimer's parasite which cannot possibly be identified. If a coccidium at all, it may have been an *Isospora* or an *Eimeria*, but nothing more definite can be asserted. In the case recorded by Virchow, however, we know that he was familiar with the small *Isospora* of the dog. He recognised that the latter occurred in the tissues of the villi and not in the epithelium, and that it occurred in pairs and was like the parasite of the cat described by Finck. Of the human form, he says it occurred in the interior of the villi, especially towards their distal ends, and that it agreed entirely with the one he had seen in the dog. The only possible conclusion which can be drawn legitimately from these precise statements is that Virchow actually meant what he said and was observing in man a small *Isospora* like *Isospora bigemina* of dogs and cats. With the very doubtful exception of the bodies seen by Railliet and Lucet (1890) this small coccidium has not since been discovered. At first sight this may seem surprising, but there appears to be a possible explanation. When Finck made his observations on the cat he was concerned mostly with the changes undergone by the intestinal epithelium during digestion rather than with the faeces. He was actually examining the intestinal wall itself, and not the dejecta of his animals. Similarly, Virchow and Rivolta, who saw the small *Isospora* of dogs, were concerned mostly with the wall of the intestine, and the same appears to be true of Railliet and Lucet, and Stiles. As pointed out above, the presence of *Isospora bigemina* in the cat was only detected by the writer when scrapings were made from the intestinal wall. In these scrapings the thick-walled sporocysts, often arranged in pairs enclosed by a common membrane, were very striking objects, whereas the incompletely formed oocysts of the large *Isospora felis* which were also present were not nearly so easily seen, and might readily have been mistaken for enlarged tissue cells. If examination in this case had been limited to the faeces the small forms would have been missed entirely, and only the oocysts of the large form seen.

Grassi, however, was concerned largely with the examination of the intestinal contents and faeces, with the result that he discovered the oocysts of the intermediate sized *Isospora rivolta* in the cat.

When he examined the intestinal epithelium he noted that they were present in the epithelial cells, but there was no indication of a paired arrangement as in the case of the small *Isospora bigemina* seen by Finck and others. Since Grassi's time, Wasielewski and other observers, who have likewise studied the faeces, have noted in cats and dogs both *Isospora rivolta* and *Isospora felis*, but never the small *Isospora bigemina*. The developmental stages of the larger forms have been seen only in the epithelial cells, and never in the paired condition in the tissues of the villi. It is not improbable that the tissue-invading small form has been frequently missed owing to failure on the part of investigators to examine scrapings from the intestinal wall itself. Virchow discovered the small form in man because he adopted this method, and it is probable that it would have been re-discovered in recent years had this practice been continued and if examinations had not been limited to the faeces alone.

During the extensive examination of faeces of men necessitated by the exigencies of the war, the oocysts of an *Isospora* were discovered on many occasions. They were first seen by Woodcock (1915) and then by the writer (1915), who demonstrated their development and proved that they actually belonged to the genus *Isospora*, as had been suggested by Woodcock. In a recent paper, Connal (1922) has shown that over one hundred and fifty cases of infection with this parasite are on record. The oocysts measure from 25 to 30 microns in length by about 12 to 15 in breadth. They thus correspond in size with those of *Isospora rivolta* of cats and dogs. They differ, however, in shape, so that they cannot be identified with the parasite of dogs and cats. From what has been said above, it is evidently impossible to identify this human *Isospora* with the small form (*Isospora bigemina*) of cats and dogs or with the small form (*Isospora hominis*) seen by Virchow in man. The fact that the oocysts appear in the stool in the undeveloped condition is strongly suggestive of a development in the epithelium like *Isospora rivolta* and *Isospora felis* of cats and dogs.

Dobell (1919), in his careful review of the coccidia of man, based his arguments on the assumption that only one *Isospora* occurred in cats and dogs, and under the name *Isospora bigemina* he included the small, intermediate and large-sized forms of these animals.

Hence in his discussion of the name which should be applied to the *Isospora* of man, with every justification he included under the name *Isospora hominis* the small form described by Virchow and the much larger form discovered during the war. When it is realised that the small form in cats and dogs which develops in the tissues of the villi is distinct from the larger forms which develop in the epithelium, this position as regards the human parasites at once becomes untenable. The small *Isospora* of man described by Virchow was named *Isospora bigemina* var. *hominis* by Railliet and Lucet (1891), a name which becomes *Isospora hominis* Railliet and Lucet, 1891. As we have seen, the name *Cytospermium hominis* of Rivolta was given to unidentifiable structures seen by Eimer (1870). Dobell (1919) recognises this latter fact, but adopts the position that it is better to assume that Eimer was actually dealing with the form described by Virchow, and strongly urges that this view be accepted. But this statement was made on the assumption that the small forms in the dog and cat were identical with the larger ones, an attitude which is maintained by Dobell and O'Connor (1921), who employ the name *Isospora rivoltae*. It seems unwise to make this assumption, as there are absolutely no data to indicate the nature of the structures seen by Eimer. It is more logical to adopt the name *Isospora hominis* Railliet and Lucet, 1901, for the small *Isospora* of man, and to regard Rivolta's name *Cytospermium hominis* as a *nomen nudum*.

As regards the large *Isospora* of man, no special name has been given to it, though Savage and Young (1917) employed the term *Coccidium isospora* for this parasite. As pointed out by Dobell (1919), this is evidently a misprint or *lapsus calami*. The intention of the writers was not to introduce a new name, but to refer to a coccidium of the genus *Isospora* in contradistinction to one of the genus *Eimeria*, as coccidia belonging to both these genera had been recorded from man during the examinations for intestinal protozoa made during the war. If, however, it is claimed that the name was correctly presented, then, *Coccidium* being a synonym of *Eimeria*, Savage and Young's name becomes *Eimeria isospora*, and one would have to conclude that they were recording an *Eimeria* of man. There is actually no evidence in the paper that this was not the case, however improbable such a conclusion may be. Their name is, strictly speaking, a *nomen nudum*.

An appropriate name for the *Isospora* of man which figured so largely during investigations on the intestinal parasites of man conducted during the war would be *Isospora belli*. It may at first sight appear to cause confusion to introduce a new name for a parasite which is now generally known as *Isospora hominis*, but Virchow (1860) so definitely referred to a small *Isospora* of man, which was named *Coccidium bigeminum* var. *hominis* by Railliet and Lucet (1891), that to submerge this form by applying the name to a much larger and evidently distinct species which is perhaps more easily detected, is not only contrary to scientific procedure, but is unfair to its discoverer and misleading to future investigators. It seems highly probable that if the method of examination of the small intestine at post-mortem by scrapings from the wall be adopted as a regular procedure the small *Isospora hominis*, first seen by Kjellberg, will be re-discovered.

#### CONCLUSIONS

1. There occur in cats and dogs three species of coccidia of the genus *Isospora*, namely, *Isospora felis* n. sp., *Isospora rivolta* (Grassi, 1879) and *Isospora bigemina* (Stiles, 1891). The last named is a small parasite of the deeper tissues of the villi of the small intestine, and development of the oocyst may be completed in the vertebrate host, while the two former are larger and are parasitic in the epithelium covering the villi, the development of the oocysts not taking place till they have left the body.
2. It is possible, as maintained by Railliet and Lucet, that there are different varieties of *Isospora bigemina*, namely, *I. bigemina* vars. *canis*, *cati* and *putori* from the dog, cat and pole cat, respectively, but there is at present insufficient evidence to justify the conclusion that they are distinct.
3. The large parasite of the 'swift fox,' described by Weidman as a possible variety of *Isospora bigemina*, does not belong to this species, but is more nearly related to *Isospora felis*. If it is a new species, its name will be *Isospora canivecolis*.
4. The complete development of *Isospora felis* in the epithelium is described. A characteristic feature of the intracellular stages is the gregariniform character of the parasite. Schizonts produce, as



a rule, eight merozoites, but sometimes a larger number. The nuclei of the microgametes are the result of repeated division of the original single nucleus of the young microgametocyte. The karyosome appears to be present in all stages of growth of the parasite. The oocyst wall is not completely formed till the parasite has left the cell, and no change in its contents occurs till the oocyst has left the body.

5. The complete development, including schizogony and sporogony, of *Isospora bigemina* takes place in large cells in the internal tissues of the villi, and here the oocyst is formed and completes its development. Its wall is comparatively thin, while that of the sporocyst is relatively thick.

6. The development of the oocyst of *Isospora rivolta* was studied, and this takes place only after it has left the body, as in the case of *Isospora felis*.

7. The parasite described from the interior of the villi of man by Virchow is a small *Isospora* like *Isospora bigemina*. It bears the name *Isospora hominis* (Railliet and Lucet, 1891).

8. For the larger form discovered in the faeces of man during the war, and regarded by Dobell as identical with the small form described by Virchow, the name *Isospora belli* n. sp., is proposed.

9. A coccidium of the genus *Eimeria* is described from the faeces of dogs. This form is remarkable in that the oocysts vary considerably in size. The name *Eimeria canis* n. sp., is proposed for this parasite.

The writer is much indebted to various people for assistance rendered; especially to Dr. G. Lavier, of the Faculté de Médecine, Paris, who very kindly obtained information regarding certain papers which could not be found in London.



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## ADDENDUM

Since the foregoing account of the coccidia of cats and dogs was written, a paper has come to hand by Zapfe dealing with the *Isospora* of dogs in Germany. The form studied appears to be the one of intermediate size mentioned by Reichenow (1921), and which has been identified as *Isospora rivolta*. It was assumed above that the development of *Isospora rivolta* would be found to take place in the intestinal epithelium, and this has been demonstrated by Zapfe. The various stages are very similar to those of *Isospora felis*, but they are correspondingly smaller, as was to be expected from the smaller size of the oocyst. During schizogony from eight to twenty-four merozoites are produced. The infection is as a rule limited to the distal ends of the villi, as in the case of *Isospora felis*. Zapfe regards the parasite as *Isospora bigemina*, and discusses the statements that have been made as to the occurrence of oocysts in the interior of the villi. He inclines to the view that the oocysts are not actually in this situation, but only appear to be there on account of irregularities in the epithelium. It is evident he has not encountered the small form which unquestionably develops in the interior of the villi.

Reference is made to a paper by Pospiech (1919), which the writer has not seen. This author examined the faeces of a large number of dogs, and came to the conclusion that there were actually four types of *Isospora* in cats and dogs. Three of these correspond with the three forms described above. A fourth type, which occurs in both cats and dogs, has an oocyst which varies in size between that of *Isospora bigemina* and *Isospora rivolta*. The dimensions are given as 17 to 18 microns by 14 microns. The size of the sporocyst is 11 by 7.5 microns. The writer has not seen this form in England, and can express no opinion as to whether it is a distinct species. Zapfe also mentions a paper by Bornhauser (1912), who described a coccidium of the liver of dogs. Nöller is quoted as having expressed the opinion that the structures described were



probably not parasites at all. Reichenow (1921) has come to the same conclusion.

A paper by Otten (1923) refers to the separation of oocysts from the faeces of dogs by a saline concentration method.

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THE HISTORY OF THE

REIGN OF

CHARLES THE FIRST

BY SAMUEL JOHNSON

IN TWO VOLUMES

LONDON: Printed by A. MILLAR, in Pall-mall.

1729.

Vol. I.

CHAP. I.

THE DEATH OF KING JAMES THE FIRST.

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## EXPLANATION OF PLATE IX

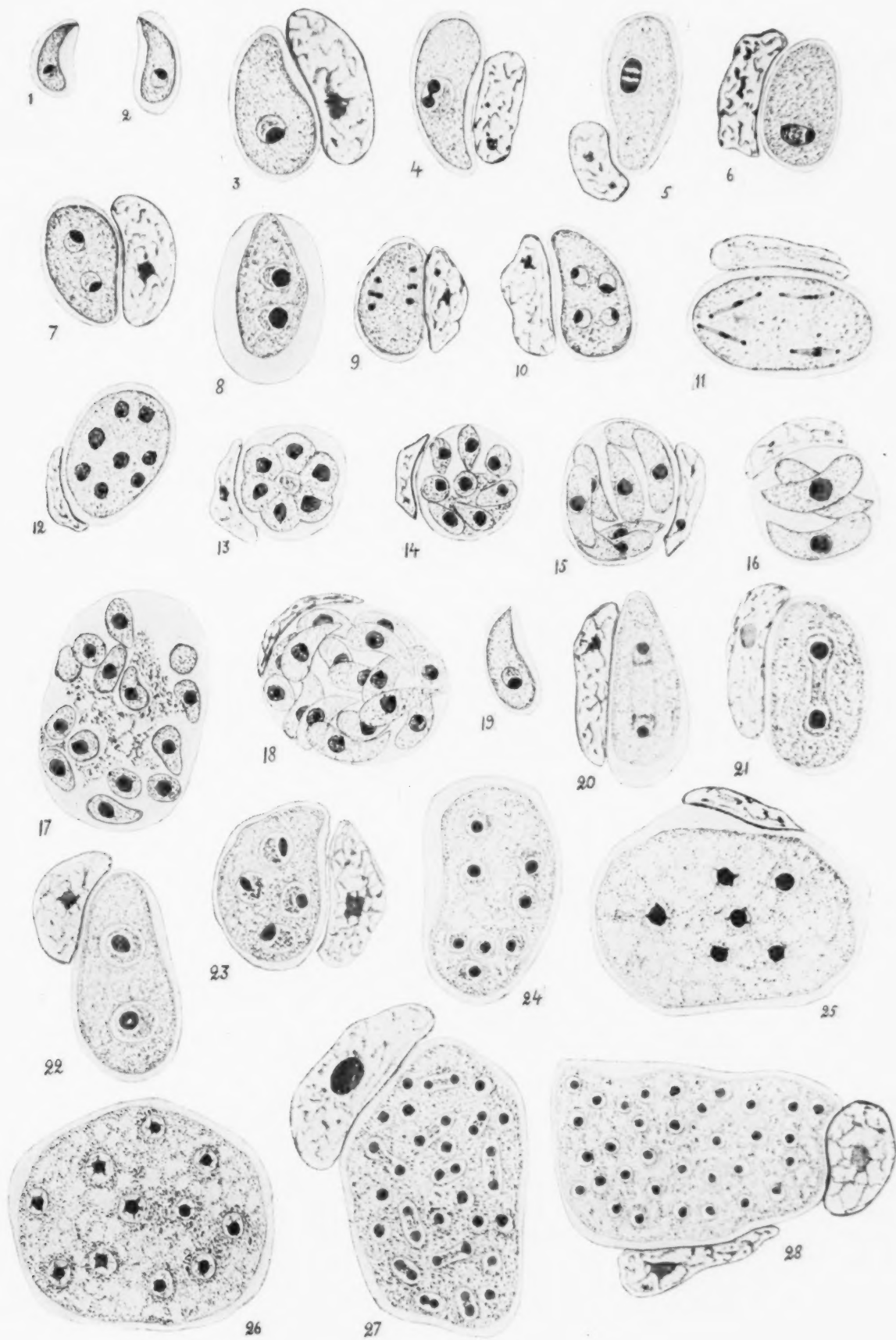
*Isospora felis.* ( $\times 2000$ .)

Figs. 1-18. Schizogony.

1. Smallest form in vacuole in epithelial cell showing attachment to surface of vacuole.
2. Slightly larger form with similar attachment.
3. Stage just prior to commencement of nuclear division.
4. Commencing nuclear division. The karyosome in division.
5. Intranuclear spindle showing equatorial plate of chromosomes and daughter karyosomes at ends of spindle.
6. Similar stage showing daughter plates of chromosomes.
7. Stage with two nuclei.
8. Similar stage.
9. Second nuclear division.
10. Stage with four nuclei.
11. Third nuclear division.
12. Stage with eight nuclei. The karyosome is still present though reduced in size.
13. Formation of merozoites from the central cytoplasmic body. Only six of the eight merozoites are shown.
14. Eight merozoites and residual body in vacuole in cell.
15. Eight merozoites of larger size in vacuole.
16. Three merozoites in a vacuole. This is either division into a small number of merozoites or the result of multiple infection.
17. Stage with sixteen merozoites, only fourteen of which appeared in the section.
18. Stage with sixteen larger merozoites.

Figs. 19-28. Growth of microgametocyte.

19. Young microgametocyte?
20. First nuclear division.
21. Similar form.
22. Stage with two nuclei.
23. Stage with four nuclei.
24. Stage with eight nuclei.
25. One section of stage with sixteen nuclei.
26. One section of stage with about thirty-two nuclei.
27. One section of stage with larger number of nuclei, many of which are dividing. The chromosomes can be detected.
28. One section of stage with still larger number of nuclei.

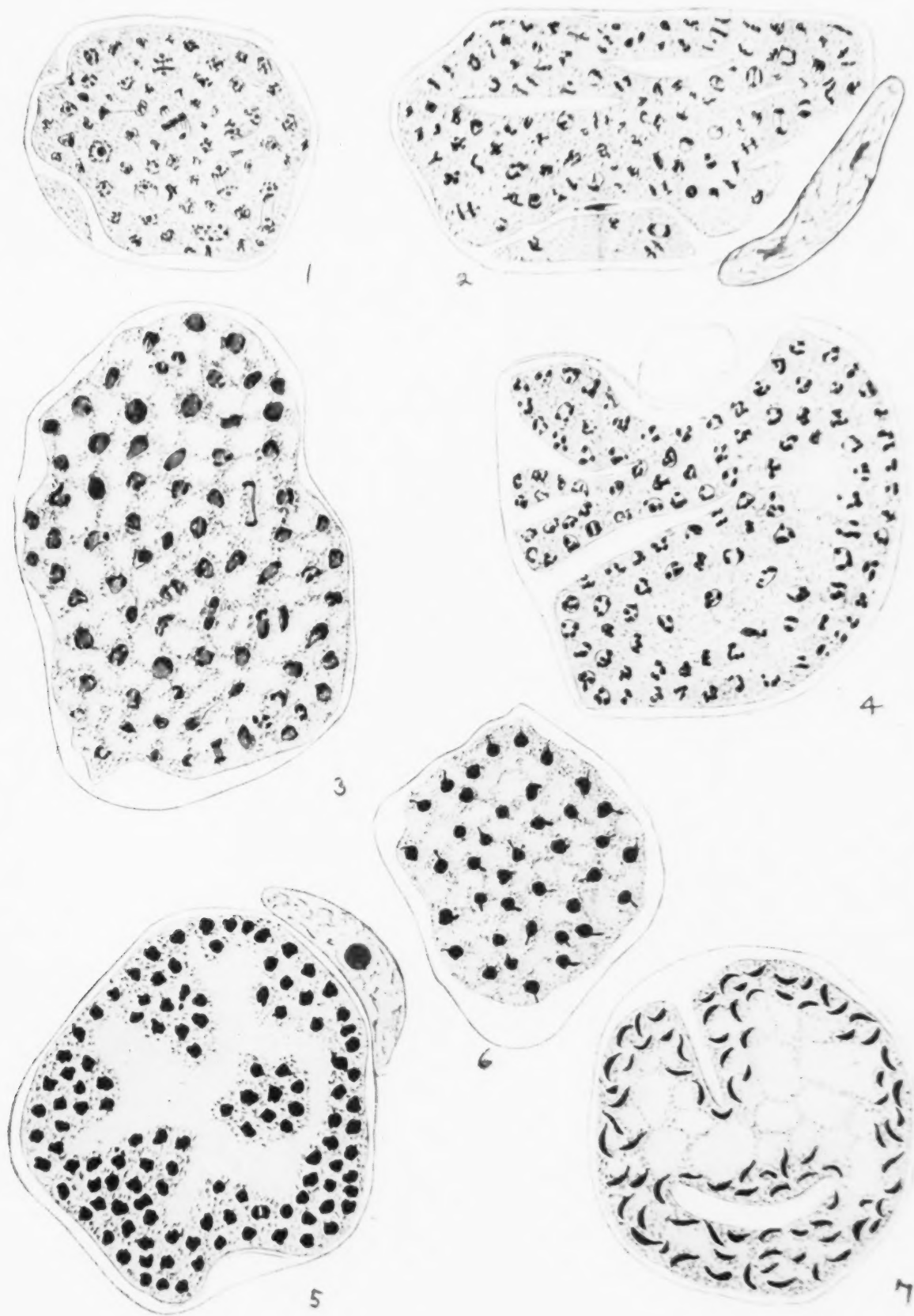


## EXPLANATION OF PLATE X

Figs. 1-7. Growth of Microgametocyte (*contd.*)

1. One section of stage in which the chromatin has become more distinct, the cytoplasm clearer and the karyosome smaller. Definite mitotic division of the nuclei is taking place.
2. One section of stage in which the chromatin is still more marked.
3. One section of stage in which the chromatin is much coarser. Some nuclei are showing what is probably the last nuclear division.
4. One section of stage in which the final nuclear division has taken place. Each nucleus includes several coarse chromatin masses. In some an isolated granule can be detected. This may be the karyosome.
5. One section of stage in which the chromatin granules are becoming aggregated into a single mass.
6. One section of stage in which the chromatin of the nuclei has become completely condensed into a single mass and has formed finger-like outgrowths.
7. One section of stage in which the chromatin of the nuclei has assumed a falciform shape.



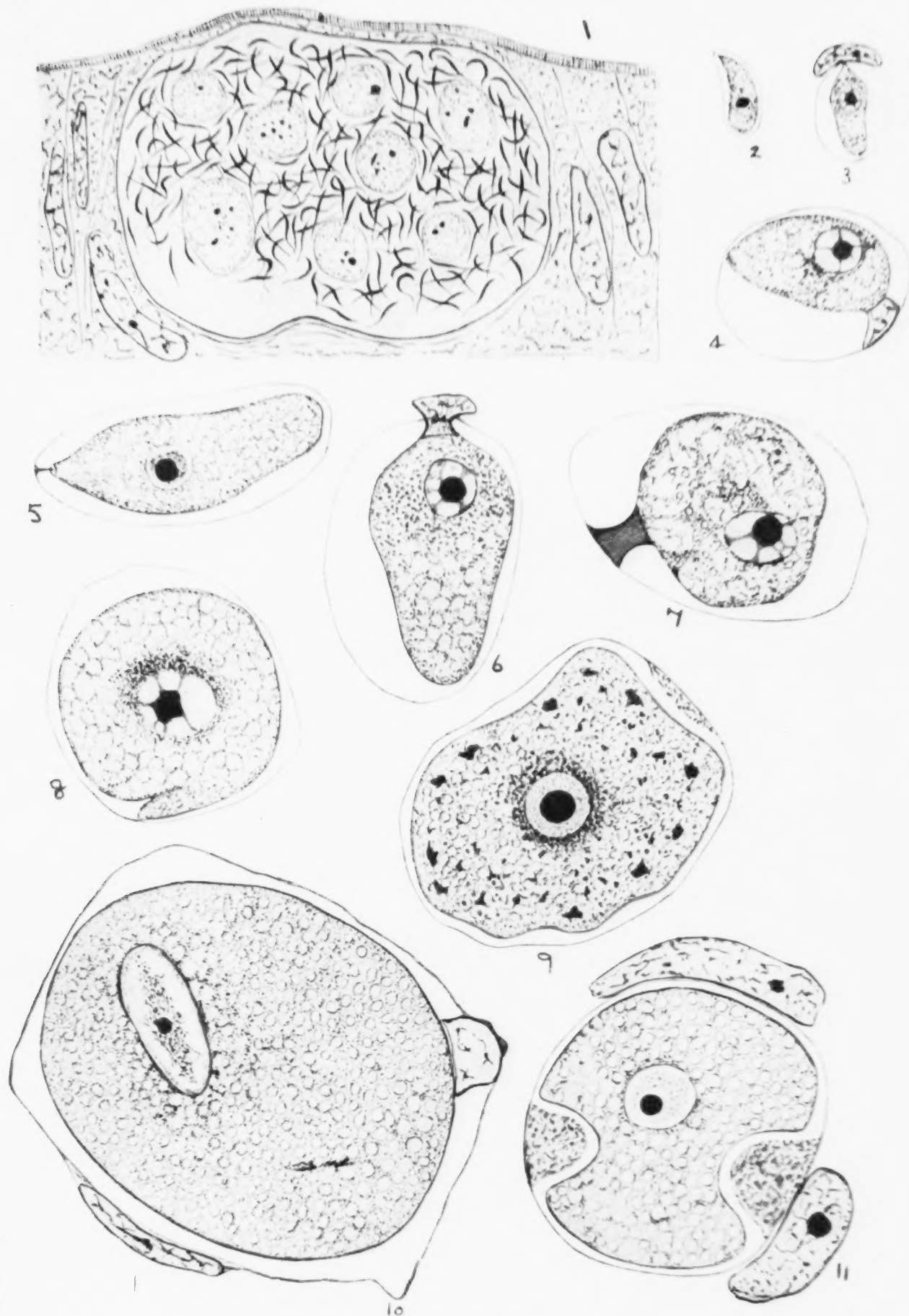


## EXPLANATION OF PLATE XI

Fig. 1. One section of stage in which microgamete formation is completed and several residual bodies are present.

Figs. 2-11. Growth of macrogametocyte.

2. Very young macrogametocyte ?
3. Slightly later stage showing attachment to the surface of the vacuole against the nucleus of the host cell.
4. Later stage showing attachment to the nucleus of the host cell, which has been drawn into the vacuole.
5. Still later stage showing the appearance of a terminal sucker into which a pedicle of the cell cytoplasm has been drawn.
6. Still later stage attached to nucleus.
7. Section of larger form with nucleus of host cell within the vacuole.
8. Section of larger form showing doubled-up condition. The granules of deeply staining material are appearing round the nucleus.
9. Section of later stage. The granules round the nucleus are more marked while deeply staining masses appear in the cytoplasm.
10. Section of larger form. Globules of a refractile substance are appearing in the cytoplasm.
11. Section of a stage in which the globules of refractile substance are more pronounced. The surface is indented in two places by an accumulation of an eosinophile granular material against the wall of the vacuole.



## EXPLANATION OF PLATE XII

Fig. 1. Fully developed stage with clear cytoplasm filled with globules of refractile substance. The oocyst wall is just commencing to form.

Figs. 2-11. Sporogony.

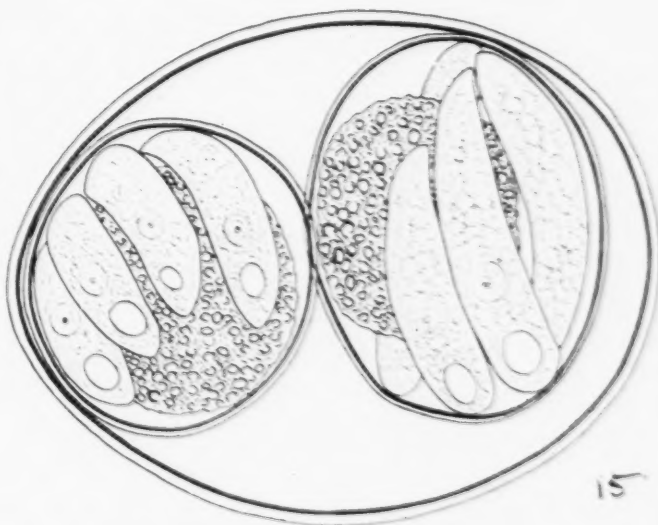
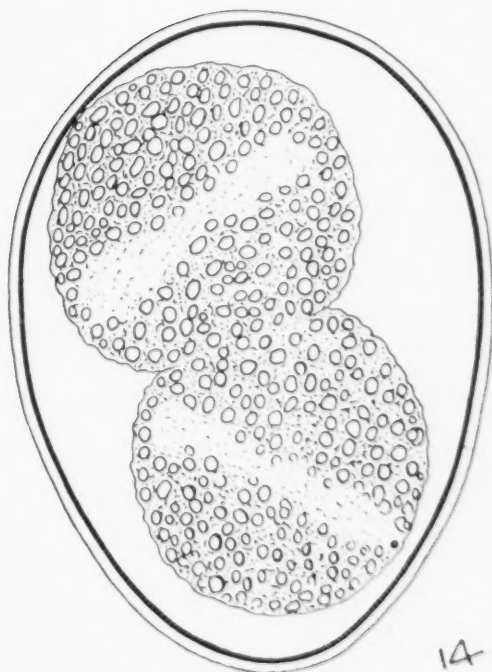
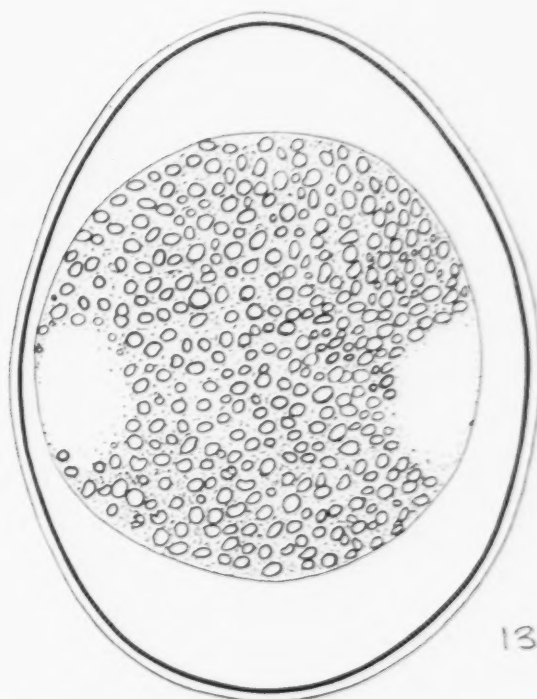
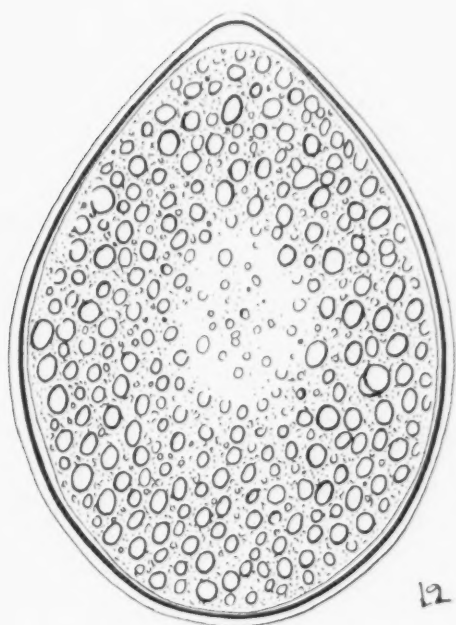
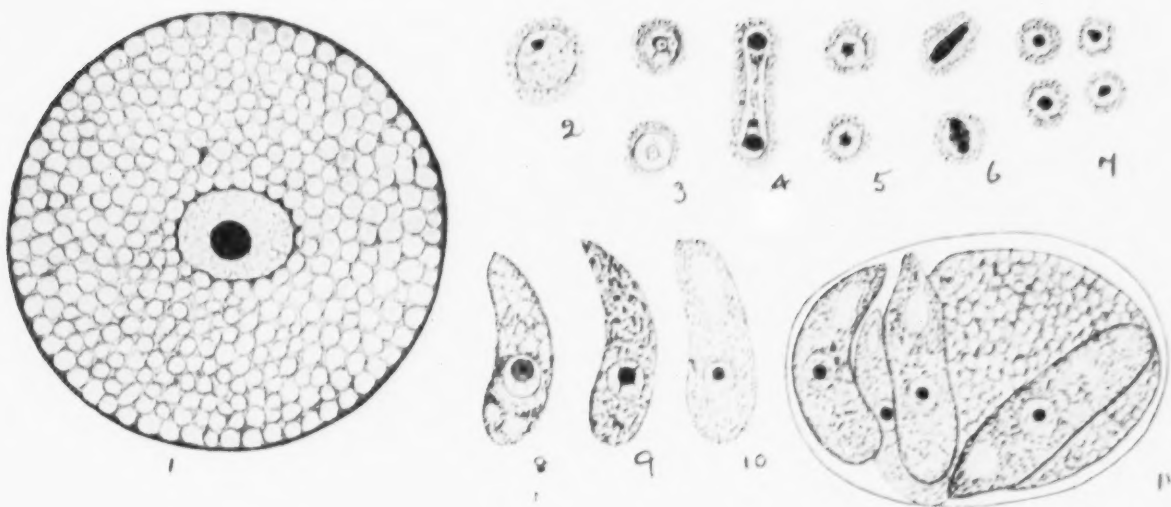
A small area of cytoplasm is figured round each nucleus in Figs. 2-7.

2. Nucleus of the zygote.
3. Two nuclei in zygote after first nuclear division.
4. First nuclear division in a sporoblast.
5. Two nuclei in a sporoblast.
6. Second nuclear division in a sporoblast.
7. Four nuclei in a sporoblast.
8. Sporozoite showing granule at centre of karyosome.
9. Sporozoite with karyosome more deeply stained.
10. Sporozoite showing vacuole in cytoplasm left by solution of refractile body.
11. Stained sporocyst showing four sporozoites and large residual body.

Figs. 12-15. *Isospora felis*—oocysts as seen in living condition. ( $\times 1500$ ).

12. Condition in which oocyst leaves the body.
13. Oocyst in which the zygote has become spherical and the nucleus divided.
14. Two sporoblasts in which first nuclear division is taking place.
15. Mature oocyst showing two sporocysts, each with four sporozoites and a residual body.







## EXPLANATION OF PLATE XIII

Figs. 1-11. *Isospora bigemina*. ( $\times 2000$ ).

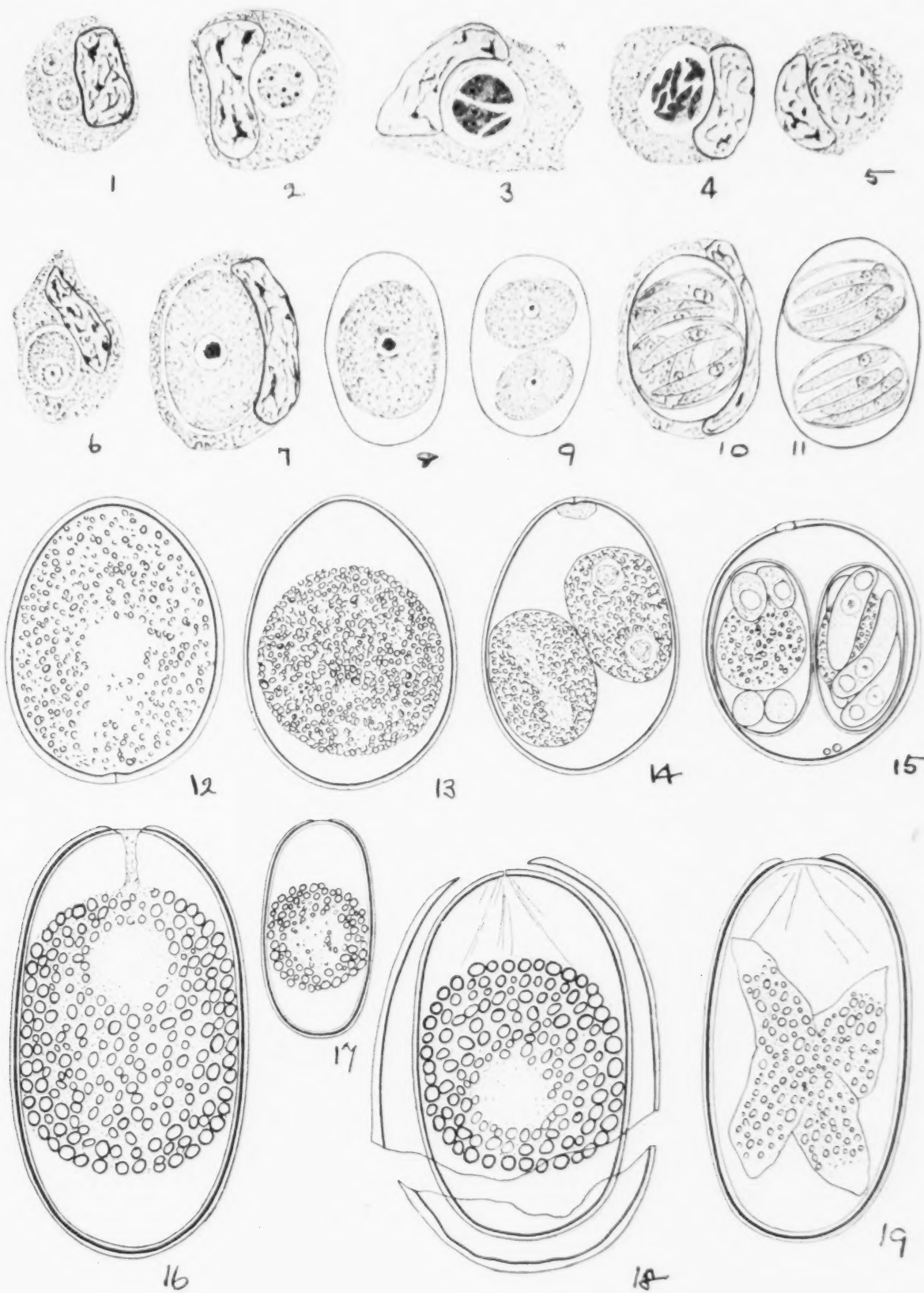
1. Two young schizonts in mononuclear cell.
2. Multinucleated schizont.
3. Commencing segmentation of schizont.
4. One section of stage with about sixteen merozoites.
5. Microgametes and residual body.
6. Partly developed macrogametocyte.
7. Fully grown macrogametocyte.
8. Oocyst with enclosed zygote.
9. Oocyst with two sporoblasts.
10. Fully developed oocyst with two sporocysts, each with four sporozoites and a residual body.
11. Similar stage with no residual body visible in the sporocysts.

Figs. 12-15. *Isospora rivolta*—oocysts as seen in living condition. ( $\times 1500$ ).

12. Condition in which oocyst leaves the body.
13. Oocyst in which zygote has become spherical.
14. Oocyst with two sporoblasts in one of which the nucleus is dividing, while in the other the first nuclear division is complete.
15. Mature oocyst containing fully developed sporocysts.

Figs. 16-19. *Eimeria canis*—oocysts as seen in the living condition. ( $\times 1500$ ).

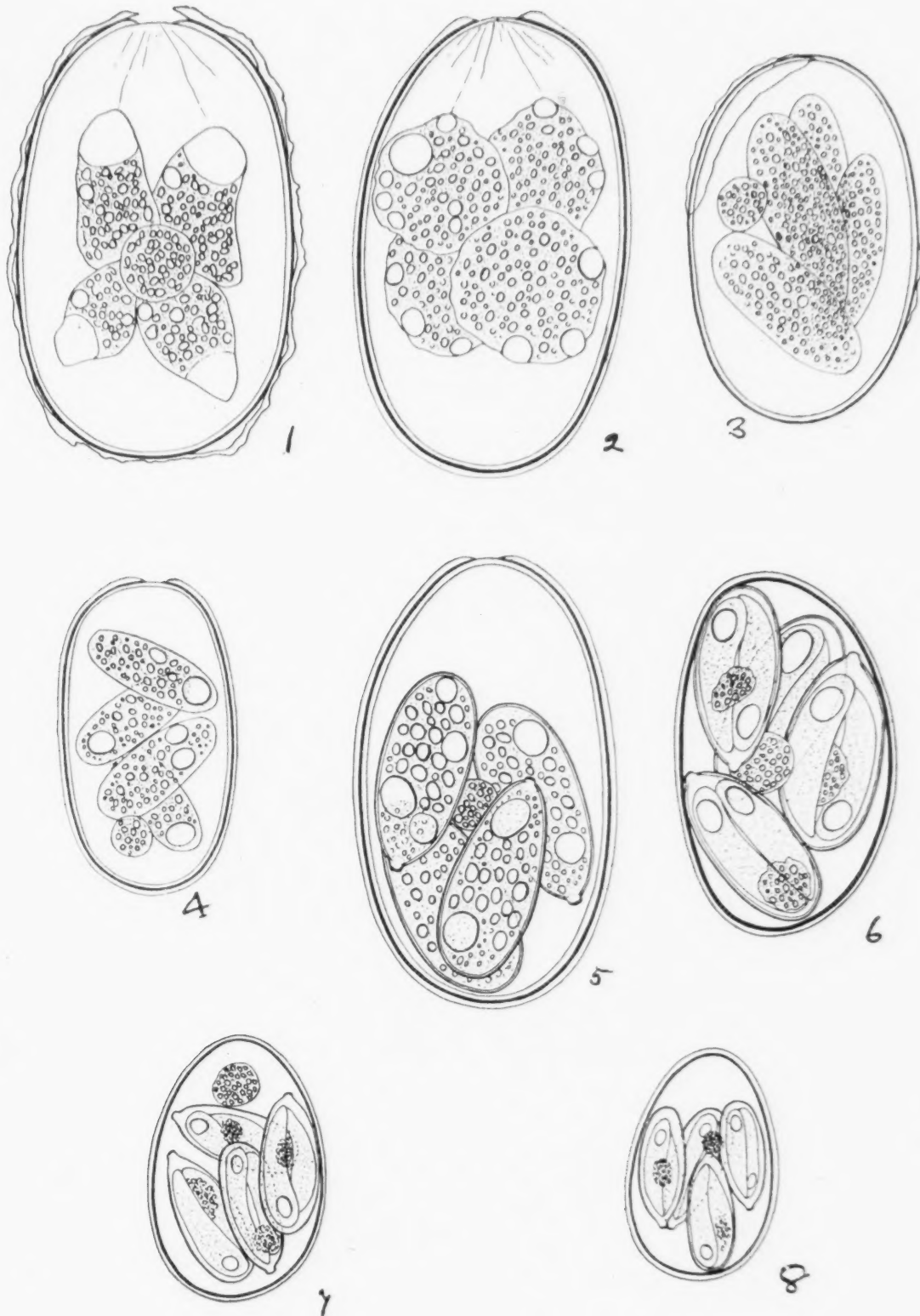
16. Large oocyst with spherically contracted zygote attached to micropyle by pedicle.
17. Very much smaller oocyst of similar type.
18. Large oocyst with the outer covering breaking away.
19. Oocyst with outer covering intact. The zygote is budding off from sporoblasts as pyramidal bodies.



## EXPLANATION OF PLATE XIV

Figs. 1-8. *Eimeria canis*—oocysts as seen in the living condition (*continued*).  
( $\times 1500$ )

1. Oocyst with outer covering intact. Four sporoblasts and a residual body are present.
2. Similar form with four sporoblasts and no residual body.
3. Oocyst with four elongated sporoblasts and a residual body. The outer covering of the oocyst has disappeared except at two small areas.
4. Oocyst in similar stage of development with outer covering intact.
5. Oocyst with outer covering intact and four undeveloped sporocysts and a residual body.
6. Completely developed oocyst with residual body and four sporocysts, each of which has a terminal knob and includes two sporozoites and a residual body.
7. Completely developed oocyst of much smaller size.
8. Similar but slightly smaller oocyst.







# A FURTHER NOTE ON THE OCCURRENCE OF ANCYLOSTOMES RESEMBLING *NECATOR AMERICANUS* AMONGST DOMESTIC PIGS IN AMAZONAS

BY

R. M. GORDON

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In a previous note (1922) the author gave a brief description of Necators obtained from domestic pigs in Amazonas and reached the conclusion that, although of smaller size, the worm was indistinguishable from the human parasite *Necator americanus*. About the same time Ackert and Payne (1922) described a hookworm from the gut of the domestic pig in Trinidad. They stated that although this worm resembled *Necator americanus*, it exhibited certain differences which in their opinion were of specific value and accordingly they gave it the name *Necator suillus*. They elaborated their arguments in a later paper (1923). The points on which they base their conclusion that the pig worms differ from the human are the following:—

- (1) 'The new species is somewhat smaller.'
- (2) 'The buccal capsule much smaller proportionately.'
- (3) 'As a rule the dorsal turn in the neck is not so pronounced as in *Necator americanus*.'
- (4) 'In *N. suillus* the lateral lancets are broadly wedge-shaped in profile, while those of the large Necator are cusp-shaped. The ventral lancet is slender in side view pointing towards the base of the dorsal tooth in *N. suillus* while this lancet in the larger species is broader and points approximately towards the tip of the dorsal tooth.'
- (5) 'The dorsal rays in the new species are shorter, while their

terminal branches are actually longer than these structures in the larger *N. americanus*.'

(6) 'In comparing specimens of these two species, cleared in glycerine, one is struck by the large so-called body cavity in the females of *N. americanus*, as contrasted with a much smaller one in *N. suillus*.'

(7) 'Concerning the males of these two species, the most striking differences are the proportions and shape of the bursa when closed. In *N. americanus* this organ is about as long as wide, and is distinctly funnel-shaped, the distal edges being flared like the bell of a trumpet, while the bursa of *N. suillus* is distinctly longer than wide and is more cup-shaped.'

(8) 'A conspicuous difference between these two species seen under higher magnification is the form of the head papillae. In *N. suillus* each lateral papilla unites with the corresponding dorso-lateral one, enclosing a conspicuous cam-shaped depression, a condition not true of *N. americanus*. Further it may be noted that in *N. americanus* the distal ends of the dorsal, lateral and ventral papillae are more or less beaded or constricted, while in *N. suillus* no such structures occur at the ends of the papillae.'

(9) 'Another rather constant difference is the shape of the externo dorsal ray. In *N. suillus* this ray, which is of nearly equal width throughout its length, makes a sharp lateral turn near its distal end; while in *N. americanus* the width of this ray is variable and the turn at the tip is less pronounced.'

(10) 'Finally the spicules show constant differences. The average length of the spicules of *N. americanus* is double that of the spicules of *N. suillus*. In the latter species both shafts terminate distally in the membranelle as recurved hooks, while in *N. americanus* only one shaft ends as a recurved hook, the other terminating in a nearly straight line.'

In view of the work of Ackert and Payne the writer has re-examined the pig ancylostomes from Amazonas and compared them with ancylostomes obtained from the human host in Amazonas and Jamaica, with special attention to the points mentioned above.

(1) *Length of the two worms.* Table I shows that, whereas the average size of the pig ancylostome is distinctly smaller, yet its maximum length is equal to the minimum length of the human parasite from Jamaica, and greater than that of the human parasite from Amazonas. The length cannot therefore in itself be used as a distinguishing character.

TABLE I.

Showing the lengths of Necators obtained from pig and human hosts.

	Males				Females			
	Number measured	Maximum length in millimetres	Minimum length in millimetres	Average length in millimetres	Number measured	Maximum length in millimetres	Minimum length in millimetres	Average length in millimetres
From pig, Amazonas ...	28	6.5	4.5	5.1	64	8.2	5.5	6.5
From human host, Jamaica ...	28	9.0	6.5	7.8	64	13.0	8.5	10.9
From human host, Amazonas ...	28	8.0	5.0	6.8	64	11.5	7.5	9.1

(2) *Size of buccal capsule.* It appears from Table II that the buccal capsule is proportionately greater in the pig than in the human ancylostome; this is the reverse of Ackert and Payne's findings. Much reliance cannot, however, be placed on small differences in these measurements, as well-marked variations in shape from the normal oval of the

TABLE II.

Showing the measurements of the buccal capsules in Necators obtained from pig and human hosts.

	Males							Females						
	Number measured	Maximum		Minimum		Average		Number measured	Maximum		Minimum		Average	
		Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.		Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.
From pig, Amazonas...	14	$\mu$ 66	$\mu$ 59	$\mu$ 59	$\mu$ 51	$\mu$ 60	$\mu$ 50	31	$\mu$ 81	$\mu$ 74	$\mu$ 66	$\mu$ 59	$\mu$ 68	$\mu$ 57
From human host, Jamaica ...	15	74	57	47	44	59	50	15	85	68	57	54	70	61
From human host, Amazonas ...	15	64	61	51	44	61	46	15	85	68	64	47	74	60

mouth capsule were frequently encountered; another difficulty is that any variations in the angle from which the head is viewed will give rise to different results in the measurement of the mouth capsule.

(3) *Dorsal curvature of anterior part of body.* No such constant differences as those described by Ackert and Payne were observed in the anterior curvature.

(4) *Ventral lancets, lateral lancets and dorsal tooth.* An examination was made of a large number of worms from both pig and man, but no constant differences in the lancets or dorsal tooth were found; the dorsal tooth and the ventral and lateral lancets of both worms showed great variation in size, shape, and angle of projection, as is illustrated in fig. 1,

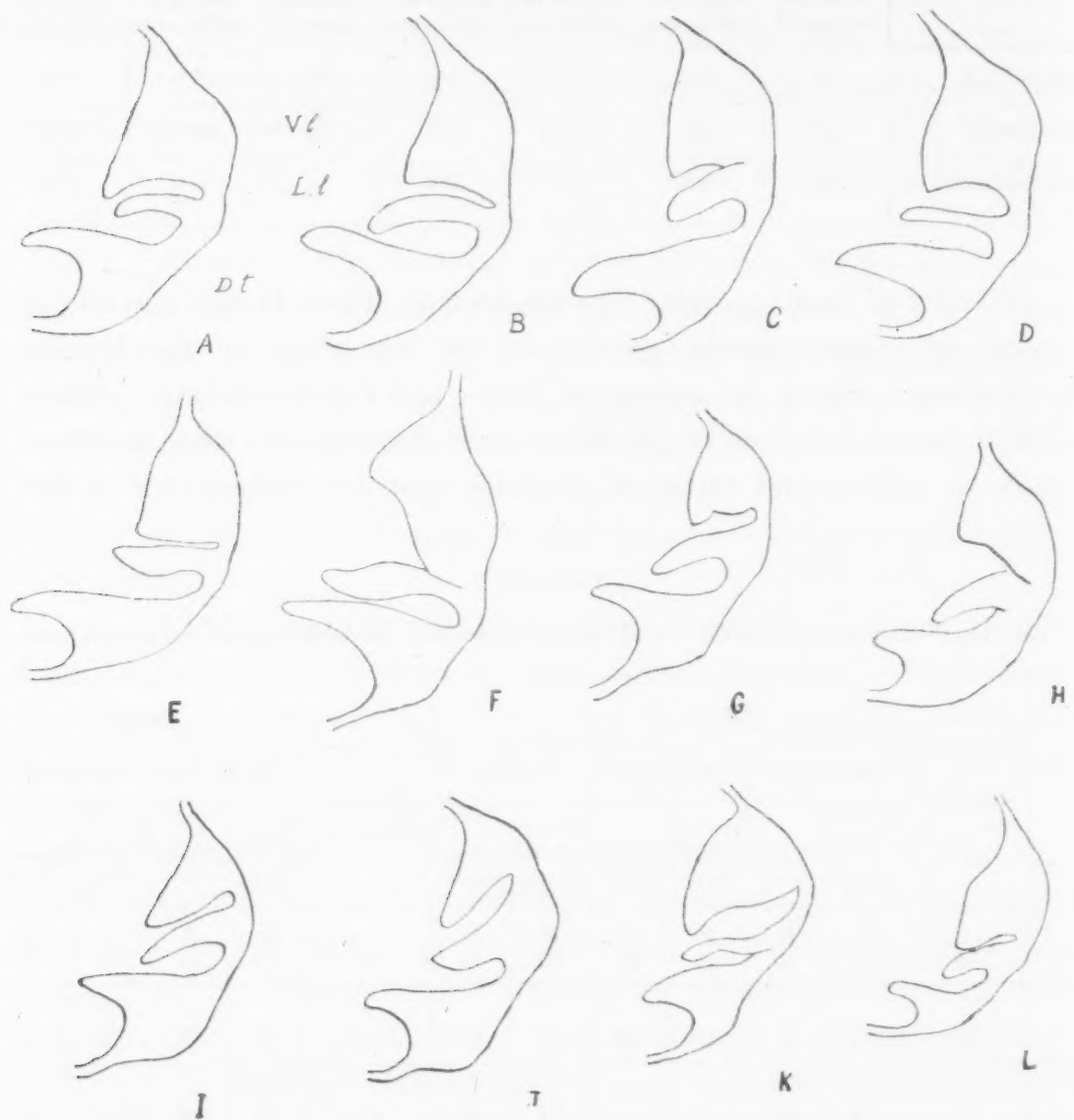


FIG. 1. The three projections in each drawing from above downwards are *V.l.*=Ventral lancet; *L.l.*=Lateral lancet; *D.t.*=Dorsal tooth. *A, B, C, D, E* and *F*=*Necator americanus* from human host, Amazonas; *G, H, I, J, K* and *L*=*Necators* from domestic pig, Amazonas.

which represents camera lucida outlines drawn from unselected material. With regard to the angle of projection of the ventral lancets it is of interest to note that Ackert and Payne in their last paper depict these lancets as projecting at almost precisely the same angle in both *N. americanus* and *N. suillus*.



(5) *Length of the dorsal rays.* The results of measuring the dorsal rays in eighteen worms from pigs and in twenty-four from human material are shown in Tables III and IV.

TABLE III.

Showing measurements of dorsal rays and their branches in *Necators* obtained from pig and human hosts.

	Length in microns of dorsal ray			Length in microns of inner branch of dorsal ray			Length in microns of outer branch of dorsal ray		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
From pig, Amazonas ...	95.0	65.0	80.0	34.0	18.0	26.4	16.0	10.4	13.9
From human host, Jamaica .....	130.0	97.0	111.5	36.4	26.0	29.9	19.0	11.7	14.3
From human host, Amazonas ... ..	120.0	78.5	110.5	32.5	19.5	25.9	15.0	7.8	11.8

TABLE IV.

Showing ratio of length of dorsal ray and its branches to total length of the worm in *Necators* obtained from pig and human hosts.

	Ratio of length of dorsal ray to total length of worm			Ratio of length of inner branch of dorsal ray to total length of worm			Ratio of length of outer branch of dorsal ray to total length of worm		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
From pig, Amazonas ...	1 : 57	1 : 92	1 : 71	1 : 183	1 : 333	1 : 215	1 : 343	1 : 528	1 : 402
From human host, Jamaica ... ..	1 : 59	1 : 80	1 : 69	1 : 206	1 : 307	1 : 257	1 : 394	1 : 726	1 : 538
From human host, Amazonas ... ..	1 : 51	1 : 89	1 : 65	1 : 224	1 : 357	1 : 277	1 : 414	1 : 1023	1 : 610

From Table IV it follows that the average of the ratios of the lengths of the branches of the dorsal ray to that of the worm is slightly greater in the case of the pig *Necators* than in the human, but the maximum and minimum values overlap to such an extent as to make this point of no specific value. From Table III it can be seen that Ackert and Payne's statement that the actual lengths of the branches of the dorsal ray are greater in *N. suillus*, does not hold good for the Amazonas material.

(6) *Size of the body cavity.* Eight worms from the pig contrasted



with eight *N. americanus* from the human host in Brazil, showed no proportionate difference in the size of the body cavity.

(7) *The shape and proportions of the bursae in the two worms.* The dimensions of the closed bursa from its ventral aspect, of six *Necators* from the pig were 0.26 mm. long by 0.24 mm. broad, while those of six *Necators* from the human host in Brazil were 0.38 mm. long by 0.41 mm. broad. It is difficult to say when a bursa is completely closed and various stages between cup and funnel shape were observed both amongst *Necators* from the human and from pig hosts, but these variations in shape appear to depend entirely on the degree of approximation of the two halves of the bursa.

(8) *The head papillae.* The union of the lateral and dorso-lateral papillae as described by Ackert and Payne was clearly visible in the majority of the pig specimens, in others, however, no such union could be traced; it was, moreover, also present in many of the *Necators* of man, though possibly not as often as in those of the pig. The beading on the papillae was also found to be a variable factor and was seen at times in both worms.

(10) *Length and shape of the spicules.* The results of measuring the spicules of twelve worms from pigs in Amazonas and of those obtained from the human host in the same locality are recorded in Table V.

TABLE V.

Measurements of the spicules of *Necators* obtained from pig and human hosts in Amazonas.

	Length of worm in millimetres			Length of spicules in millimetres			Ratio of length of spicules to total length of worm		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
From pig, Amazonas ...	6.0	4.5	5.3	0.65	0.38	0.47	1 : 8.4	1 : 13.9	1 : 11.2
From human host, Amazonas ...	8.7	6.5	7.3	0.98	0.82	0.91	1 : 7.1	1 : 10.6	1 : 8.1

It appears from Table V that the average length of the spicules in the worms from man are nearly double the length of those in the worms from the pig. The average of the ratios of the length of the spicules to that of the body are, however, respectively 1 : 8 and 1 : 11, and it must moreover be noted that the maximum ratio in the case of the pig (1 : 8.4) is greater than the minimum ratio in the case of man (1 : 10.6).

and consequently this can hardly be regarded as a reliable point of distinction. Ackert and Payne's statement that both spicules in the pig *Necator* terminate in the membranelle as recurved hooks in contrast to *N. americanus* in which only one spicule is hooked, was not found to be constantly true of the worms from Amazonas, one hooked and one nearly straight spicule being very common amongst the pig *Necators*,

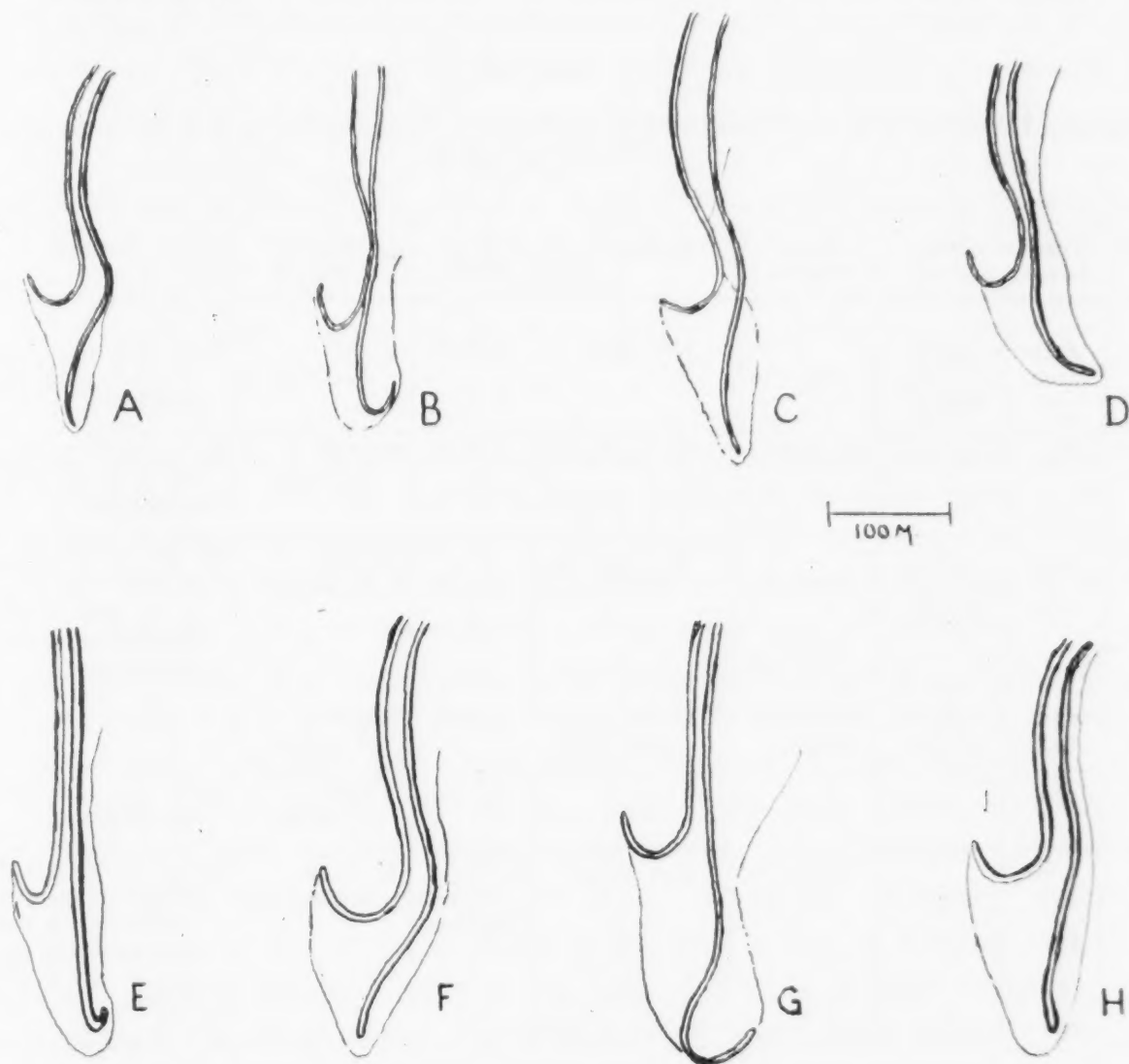


FIG. 2. A, B, C and D = Spicules of *Necators* from domestic pig, Amazonas.

E, F, G and H = Spicules of *Necator americanus* from human host, Amazonas.

while in the case of the *Necators* from man both spicules frequently showed well-marked hooks. These points are illustrated in fig. 2.

*Size of ova.* Ackert and Payne give the average size of the ova of the pig *Necators* from Trinidad as  $63\mu$  by  $37\mu$ , the ova of the pig *Necators* from Amazonas were found to measure on an average  $64\mu$  by  $39\mu$ .

ATTEMPTS TO INFECT PIGS WITH *NECATOR AMERICANUS*

Ackert and Payne (1923) performed several experiments to ascertain whether *N. americanus* from the human host can mature in pigs; they also carried out experiments in pigs with cultures of *N. suillus*. For the sake of brevity, the results of Ackert and Payne's experiments are condensed in Table VI.

TABLE VI.

Summarising the results of Ackert and Payne's attempts to infect domestic pigs with larvae of *N. americanus* obtained from human sources.

No. of pig	No. of larvae	Date given	Stage of larvae	How given	Date killed	Result of autopsy	Remarks
1	2841 2700 3000	16.6.21 20.6.21 27.6.21	} 'Infective'	Not stated	10.8.21	3 adult <i>N. suillus</i>	Faeces free from Nematode ova or larvae at time of experiment
2	2841	16.6.21					
3	1850 12000 4000	27.9.21 29.9.21 4.10.21	} 'Infective'	By mouth on bread	3.12.21	7 adult <i>N. suillus</i>	In spite of oil of Chenopodium treatment, pigs 3, 4, and 5 all had Ancylostome ova at time of experiment
4	7500 9900	29.9.21 1.10.21					
5	Nil	—	—	—	3.12.21	3 adult <i>N. suillus</i>	Used as a control for pigs 3 and 4
6	5000		'Infective'	Not stated	73 days after infection	No hook-worms found	Owing to the difficulty of obtaining pigs free from ancylostomes in Trinidad, this experiment was conducted in Manhattan, Kansas

Ackert and Payne claim to have shown from these experiments that it is not possible to infect pigs with *Necator americanus*, either by the mouth or by the skin. This claim appears to the writer to rest entirely on their ability to distinguish between *Necator americanus* and *Necator suillus*, for in every experiment, except in the case of Pig 6, where the method of administering the larvae is not stated, *N. suillus* was found at the autopsy. It is true that the number of worms found is small (3 to 8),

but on consulting Table VII, it will be seen that equally large doses (6,900 and 23,000) of infective larvae of *N. suillus* resulted in an almost equally small production (10 to 32) of adult worms. It appears certain

TABLE VII.

Results of Ackert and Payne's experiments at infection of domestic pigs with larvae of *N. suillus* obtained from naturally infected pigs.

No. of pig	No. of larvae	Date given	Stage of larvae	How given	Date killed	Result of autopsy	Remarks
1	2500 4400	29.9.21 1.10.21	} 'Infective'	By mouth	Died 5.11.21	32 <i>N. suillus</i>	Pigs 1 and 2 both showed ancylostome ova in their faeces previous to the experiment for which they received anti helminthic treatment the results of which are not recorded
2	11000 12000	9.10.21 11.10.21		Placed on shaven skin	3.12.21	10 adult <i>N. suillus</i>	

therefore that it is extremely difficult to infect the domestic pig with Necators whether the infective larvae used are obtained from other pigs or from the human host.

The present writer while in Amazonas undertook no experiments to infect pigs, owing to the difficulty of obtaining pigs free from Necators and to his inability to distinguish between the pig and the human Necators. One experiment has since been carried out in Liverpool. A pig six weeks old was obtained and kept under observation for seven days; during this period its faeces were examined daily by the saturated salt method (Willis, 1921), but with negative results. On 15.12.22 approximately 400 sheathed larvae obtained from a culture of human faeces were mixed with 2 c.c. of Normal saline and injected subcutaneously in the back of the pig, a similar dose was again given on 29.12.22. The patient from whom these cultures were made was later treated with Carbon tetrachloride and all the 96 worms obtained proved to be *N. americanus*, it therefore appears reasonably certain that the larvae administered to the pig were those of *Necator americanus*. *Trichuris* ova made their appearance in the faeces shortly after the first inoculation and persisted throughout the experiment; no other ova were seen till 19.1.23, when ancylostome-like ova first appeared in the faeces. These ova which were very regular in contour and size, measured  $68\mu$  by  $37\mu$ , and were quite indistinguishable from those



of the human ancylostomes ; they were always very scanty in numbers, never more than three being found in a single cover slip preparation made from about two grammes of faeces treated by the saturated salt method. Ancylostome ova were not always found at these examinations, the faeces being sometimes negative for two, or even three days. The last occasion on which ova were found present was 5.2.23, examinations on the subsequent four days being negative. The pig was killed 9.2.23, and in spite of a very careful search of the oesophagus, stomach, intestines, trachea, bronchi, etc., the only helminths found were numerous *Trichuris* in the caecum. During the last week of the experiment the whole bulk of the faeces was daily examined for any Ancylostomes that might be passed per rectum, this search proved negative, but it is extremely difficult to detect an odd Ancylostome in a large mass of faeces and it is therefore uncertain whether the pig got rid of the worms responsible for the ancylostome-like ova during the four days prior to the autopsy, or whether they were missed at the post-mortem ; as the search of the organs was undertaken with great care the former conjecture appears the more likely.

#### CONCLUSION

No constant differences were found between the *Necators* of Amazonas pigs and those of man from Amazonas and Jamaica such as would justify the formation of a new species for the pig worm.

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## A CASE OF ACUTE ASCENDING PARALYSIS IN A CHIMPANZEE

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(*Sir Alfred Lewis Jones Research Laboratory, Freetown*)

(*Received for publication 15 November, 1922*)

A male chimpanzee (*Anthropopithecus troglodytes*), judged to be about four years of age, was captured by natives on the 3rd of September, 1922, near Blama, Sierra Leone.

The animal appeared healthy and showed no evidence of injury to the spine. On the evening of the 5th of September the animal was chloroformed in order to be caged, and was then sent down to Freetown. Very little chloroform was used and the animal quickly recovered from the anaesthetic.

On arrival in Freetown, on the 7th of September, two abrasions in the loins, due to the chafing of a rope, were observed; these quickly improved on the application of iodine.

On the 15th of September it was noticed that the animal refused to leave its cage and did not take food; on removal from the cage it was found that the lower limbs were completely paralysed and were colder than the rest of the body. The animal eagerly drank large quantities of milk and water, but refused solid food.

On the evening of the 17th of September the trunk and the upper limbs were completely paralysed; the face was cyanosed and the animal suffered from dyspnoea. Milk feeds were vomited. The muscles of the neck were not affected and the animal could move its head freely from side to side.

On the 18th of September 0.09 gms. of Novarsenobillon were administered intramuscularly into the thigh; there was no loss of

sensation and the animal moved its head vigorously from side to side and attempted to seize the hand of an assistant with its teeth. The animal's condition appeared to improve rapidly after the administration of Novarsenobillon; the cyanosis disappeared and the respiration improved, but it was still unable to swallow solid food and lived entirely on milk.

On the 19th of September the animal passed a solid motion, the first since the 14th of September. As the condition appeared improved after the injection of Novarsenobillon, a second dose of 0.09 gms. was administered on the 20th of September.

No change occurred until the 23rd of September, when general fibrillary twitchings affecting all the muscles of the body were noticed; these twitchings were controlled by an injection of one-eighth grain of morphia. They recurred on the 24th of September, on which day the animal died.

A post-mortem was performed almost immediately after death.

The liver was pale yellowish in colour, and on section showed marked fatty degeneration.

*Central nervous system.* The cerebro-spinal fluid was slightly turbid and contained a few polynuclear leucocytes. The surface of the brain and cord were congested. Pieces of the cord from the mid-dorsal and upper cervical regions, and pieces of the medulla and cerebral cortex from the motor region (upper and lower limb centres), were fixed in alcohol, embedded in paraffin and sectioned; others were sectioned without embedding. Sections were stained in toluidine and thionin blue and differentiated with alcohol. Eosin and methylene blue, Giemsa, Leishman and Ehrlich's haematoxylin were also used.

*Microscopically*, the following changes were noticed in the central nervous system:—

Many of the cells in the cord, medulla and cortex were normal and showed Nissl's granules. In the anterior and posterior horns, and in Clarke's column, a number of cells showed faintly staining protoplasm and absence of Nissl's granules, and the nucleus tended to be eccentric in some cells. Vacuolisation of the cell protoplasm was observed in a number of cells; the vacuoles varied in size from  $2\mu$  to  $6\mu$ , and from one to six were found in each cell. Single vacuoles were found in cells which did not show marked

degeneration, but were noted in large numbers only in cells where degeneration was advanced. The vessels were congested and small haemorrhages were found. Similar changes were noted in the medulla, where vacuolisation of degenerated nerve cells was more marked than in the spinal cord.

Sections of the motor cortex from the upper and lower limb centres showed engorgement of the capillaries. No haemorrhages were found and the gross cellular changes found in the medulla and cord were not seen.

Sections of peripheral nerves showed no pathological changes.

Cultures of the heart's blood were negative.

On the 18th of September 0.2 c.c. of the animal's blood were injected intraperitoneally into a *Cercopithecus campbelli* with negative result.

On the 24th of September, during the post-mortem, 3.5 c.c. of cerebro-spinal fluid were injected intraperitoneally into a *Cercopithecus campbelli*. No paralysis followed. The animal died on the 27th of October, 1922. Post-mortem examination revealed an abscess involving the whole of the upper lobe of the right lung. Smears showed the presence of a Gram-negative capsulated pneumobacillus and a Gram-negative coccus, which was isolated in pure culture.

Before the chimpanzee's illness it had shared a cage with three younger chimpanzees which remained healthy. This, in conjunction with the fact that injection of blood and cerebro-spinal fluid into *Cercopithecus campbelli* produced no paralysis, indicates that the condition was not one of acute anterior polyomyelitis.

The lapse of time between the administration of chloroform and the appearance of symptoms also indicates that delayed chloroform poisoning was not responsible for the condition. Professor Blacklock suggests that the arsenic administered may have contributed to the condition of the liver.

The case presents interest in its close resemblance to the course of acute ascending paralysis as described in human beings.

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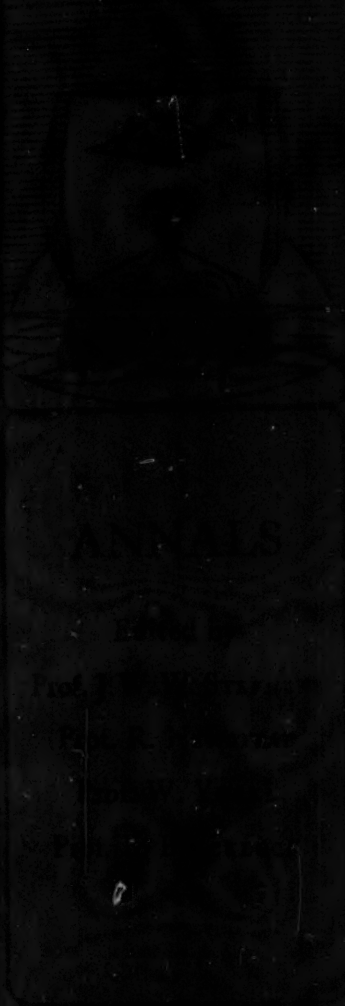
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LIVERPOOL  
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VOLUME  
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For the Treatment of

Scistosomiasis

(Schistosomiasis)

Leishmaniasis

(Kala-azar and

Oriental sore)

## ANTIMONY

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*H.R.H. The Duke of York,*  
K.G., G.C.V.O.

*Hon. President of the Incorporated  
Liverpool School of Tropical Medicine*



Albert





# STUDIES IN THE TREATMENT OF MALARIA—XXXII

## SUMMARY OF STUDIES I—XXXI

BY

J. W. W. STEPHENS

*(Received for publication 25 June, 1923)*

The results recorded in the present paper constitute a summary of work on the treatment of malaria carried out at the Liverpool School of Tropical Medicine in the years 1917-1919.

Before considering the various treatments employed, it will be necessary to define certain terms and it will be convenient also to consider certain facts which emerged as the work progressed.

### PART I

#### MALARIA

Only those cases were treated in which parasites were present in the blood at the commencement of treatment. The patient's temperature may, or may not, have been above normal.

#### RELAPSE

A *parasitic* relapse, febrile or afebrile, i.e., parasites have reappeared in the blood after a negative period induced by treatment.

#### FEBRILE ATTACKS

A rise of temperature above 100°F., unaccompanied by parasites in the blood within 2-3 days, of which the nature is unknown.

#### OBSERVATION PERIOD

If we desire to know whether a treatment has cured a patient, i.e., eliminated parasites from his system, it is obvious that the patient must be kept under observation after the treatment. The longer the patient

is kept under observation—by this we mean, not solely clinical observation, but primarily (daily) microscopic examinations of the blood for parasites, the more reasonable will it be to conclude, if the examinations are negative—that he is cured.

The period of observation employed by us was one of 60 days, implying, as we have just said, daily blood examinations. It should be unnecessary to add that *no treatment* was given during the observation period.

### FALLACIOUS FIGURES

It is necessary to point out some sources of fallacy in regard to the results of treatment, many examples of which can be found in the literature.

1. *Absence of a microscopic diagnosis of parasites in patients before commencing treatment.* Such cases may be malaria or they may not.
2. *Administration of quinine during the so-called 'observation period'.* The figures relating to relapses are obviously worthless.
3. *Comparison of treatments with different observation periods.*

If the value of two treatments are to be compared, the cases under each treatment must be observed for the *same length of time*, after the cessation of treatment, otherwise the figures for relapses are not comparable, and it is impossible to say which is the better treatment, as in the following example.

TABLE I.

Treatment	Number of cases treated	Number of cases which relapsed	Number of cases not relapsing but lost sight of before the expiration of 60 days	Number of cases not relapsing in an observation period of 60 days	Relapses	
					Actually observed	Possible maximum
I ... ..	100	10	80	10	10%	90%
II ... ..	100	30	30	40	30%	60%
III ... ..	100	50	0	50	50%	—

4. *Composite figures obtained by summarising the results of various treatments.*

The following is an example:—

Suppose two treatments employed, A and B, and that in the A treatment the relapses were 100 per cent. and that in the B treatment they were 0; and further, suppose that 750 cases were under treatment A, 250 under treatment B, then we get the following result:—

			Cases treated	Relapses	Percentage
Treatment A	...	...	750	750	100
Treatment B	...	...	250	0	0
			1000	750	75

It is correct to say that, of 1,000 cases treated, 75 per cent. relapsed.

Let us repeat the treatments and suppose that the distribution of cases treated now happens to be as follows:—

			Cases treated	Relapses	Percentage
Treatment A	...	...	250	250	100
Treatment B	...	...	750	0	0
			1000	250	25

It is also correct to say that, of 1,000 cases treated, 25 per cent. only relapsed; but the figures 25 and 75 have no *real significance*. All that is important that the figures show, is that one treatment was very good, the other very bad.

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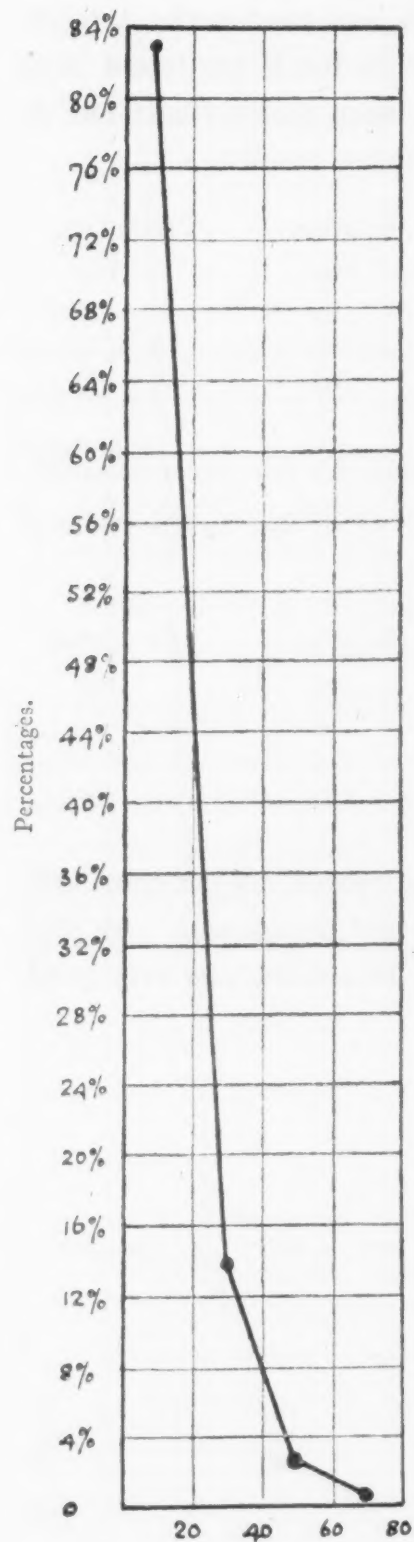
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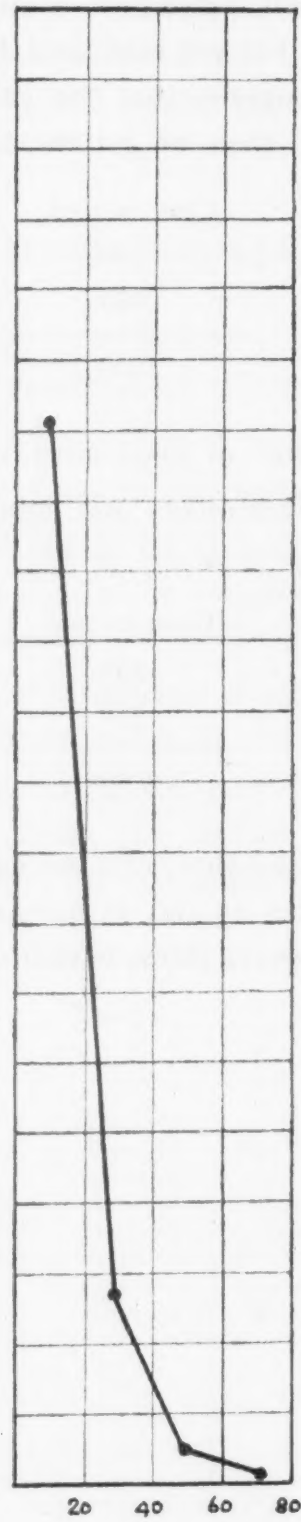
GRAPH 1.

Percentage of total relapses  
in each 20-day period.



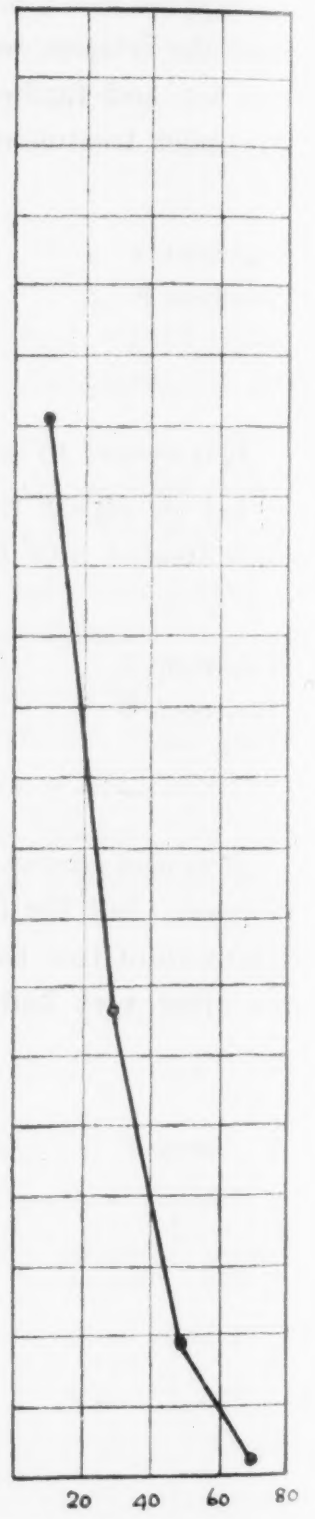
GRAPH 2.

Percentage of cases treated  
which relapse in  
each 20-day period.



GRAPH 3.

Percentage of cases treated  
not having previously  
relapsed which do so in  
each 20-day period.



Days after cessation of treatment.

### THE TIME AT WHICH RELAPSES OCCUR AFTER CESSATION OF TREATMENT IN SIMPLE TERTIAN MALARIA

The time incidence of relapses can be considered in three ways:—

1. In reference to the *relapses themselves*, i.e., the percentage of the total relapses which occur during each period of time. From an analysis of the time of occurrence of 582 relapses, we found that about four-fifths occur in the first 20 days after treatment, that the majority of the remaining one-fifth occurs in the second 20-day period, i.e., the ratio of the number of relapses in the two periods is about 4 : 1.

2. In reference to the *total cases treated*, i.e., the percentage of cases treated which relapse during each 20-day period of time. Of the cases treated (800), about three-fifths relapse in the first 20-day period, about one-tenth in the second 20-day period : still fewer at later periods, i.e., the ratio of the percentages for the two periods is 6 : 1.

3. In reference to *remainders*, i.e., the incidence among the cases treated less those who have previously relapsed. Of the cases treated (800), about three-fifths relapse in the first 20 days and about one-fourth of 'the remainder' cases in the second 20-day period. The ratios are here 12 : 5 or 2.4 : 1.

It is possible that, if a large number of cases that had not relapsed in 60 days had been observed for much longer periods, that the values we have given for the first and second 20-day periods would have to be somewhat reduced, but until the actual observations are made, this is purely conjectural.

It must be added that, unless a sufficiently large number of cases are considered, it is not likely that the ratios given above will be observed.

### TIME OF ONSET OF THE PAROXYSMS IN SIMPLE TERTIAN MALARIA

From an analysis of 1,000 'rigors' or paroxysms, we found that:—

(a) Over 90 per cent. of the paroxysms occur during the hours of bodily activity, in our series of cases from 7 a.m. to 6.59 p.m. •

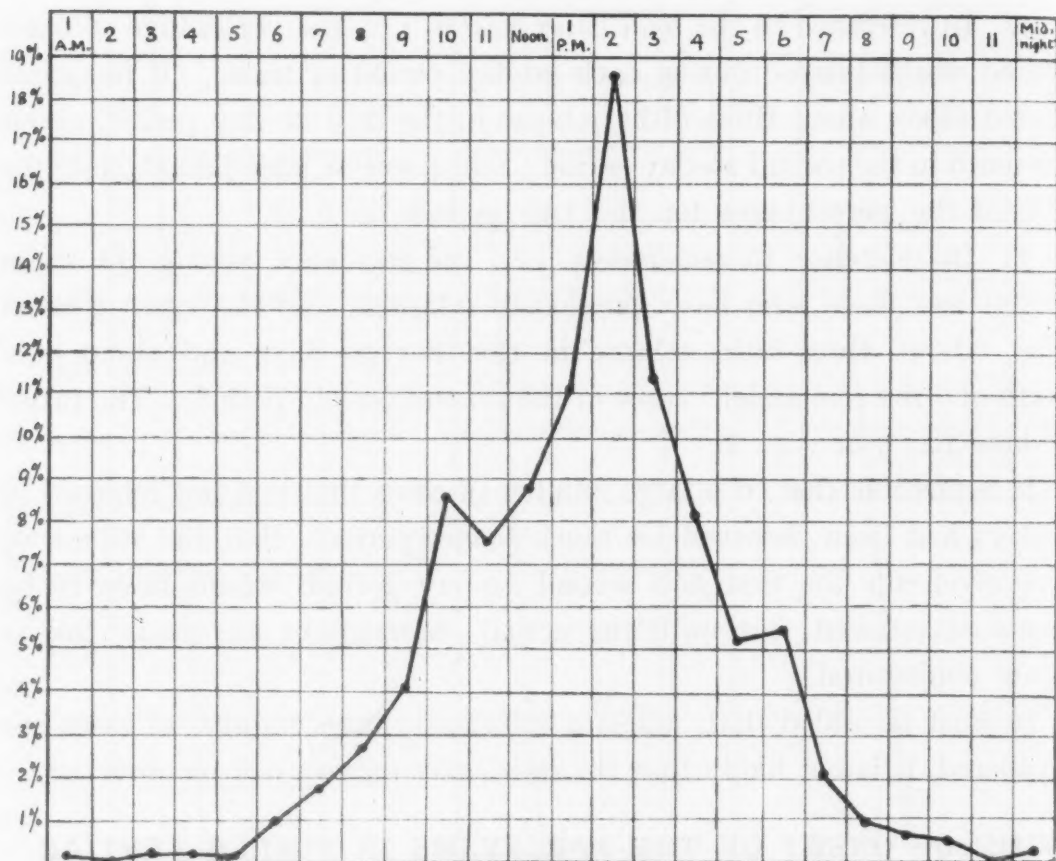
(b) The maximum number of paroxysms, about 20 per cent., occurs at 2 p.m.

### THE EFFECT OF SEASON ON TREATMENT OF MALARIA

Two series of cases consisting of 76 and 89 patients, respectively, were treated by us at different times of the year with the same treatment, viz., quinine sulphate, grains 90, on two consecutive days only.

In one series the number of relapses was about 40 per cent., in the other, over 90 per cent.

The only factor that we could discover that might account for this difference was that the cases were treated at different times of the year, the good result was obtained in the summer and autumn, the bad result in the winter and spring months.



GRAPH.—Showing the time incidence of 1,000 simple tertian malaria paroxysms ;  
'Summer' time in operation.

#### THE MAXIMUM DOSE OF QUININE THAT CAN BE TOLERATED

Quinine sulphate, orally in doses of grains 120 on each of two consecutive days, represents the maximum amount of the drug which can be tolerated by the average case, as the treatment had to be abandoned owing to severe symptoms in five of fifteen cases.

## PART II.

## TREATMENT OF AN ATTACK

## QUININE

(a) *Orally.*

Ten grains of quinine sulphate in solution on each of two consecutive days suffice to cut short an attack of simple tertian malaria, and to cause the temporary disappearance of parasites from the cutaneous blood.

While this is so, our routine procedure is to give grains 15, two to three times a day for a few days until the same result is accomplished. The subsequent treatment will be considered later.

(b) *Intramuscularly.*1. *Quinine bihydrochloride.*

Fifteen grains of quinine bihydrochloride in 2 c.c. of water on each of two consecutive days likewise cause the cessation of febrile paroxysms and effect the temporary disappearance of all stages of the parasites from the cutaneous blood. This holds good for *P. vivax* and *P. falciparum*.

2. *Quinine alkaloid.*

Grains 15 to 30 in 1 c.c. of sesame oil on each of two consecutive days, has the same effect in cases of simple tertian malaria.

Where the patient can take quinine by the mouth there is usually no necessity for intramuscular injections, but where oral quinine is ineffective, intramuscular quinine remains as a most effective treatment.

(c) *Intravenously.*

Quinine bihydrochloride in doses of 10-15 grains in a 10 per cent. solution in normal saline, in one or a series of six injections, causes the cessation of febrile paroxysms and a disappearance of parasites from the cutaneous blood in simple tertian malaria.

In *malignant tertian* malaria these doses do not cause the disappearance of parasites—trophozoites or gametes—from the cutaneous blood.

## ARSENIC

(a) *Organic. Arsenobillon.*

A single intravenous injection, 0.9 gramme, controls the fever, causes the disappearance of *P. vivax* from the cutaneous blood within 24 hours. The same dose has no appreciable effect on the temperature or the parasites in the case of *P. falciparum* or *P. malariae*.



(b) *Inorganic. Liquor arsenicalis.*

In doses of m 15 daily, failed to control the fever or to cause the disappearance of parasites. In doses of m 30 daily, the temperature fell to normal within ten days and in 13 of 14 cases parasites disappeared in two to six days.

#### SILVER ARSENIC AND ANTIMONY

*Luargol.*

A single intravenous injection of 0.2 gramme, controls the symptoms and causes the disappearance of the parasites in simple tertian malaria.

#### ANTIMONY

*Tartar Emetic.*

Intravenous injections of tartar emetic, 2 per cent. solution in one or more doses of 5-15 centigrams, do not control either the rigors or the fever of acute malaria, nor do they cause the disappearance from the blood of any stage of the malaria parasites, whether of *P. vivax* or *P. falciparum*.

#### MANGANESE

Collosol manganese 1 c.c. on each of two consecutive days proved to be valueless.

#### QUININE AND QUINOTOXIN

The hydrochlorides of these derivatives of quinine proved of no value in the doses used, viz., of about the same amount as that of quinine sulphate which proved effective.

#### AMYLOPSIN AND TRYPSIN

'Injectio amylopsini' and 'Injectio trypsin' proved to be of no value in the treatment of simple tertian malaria.



## PART III.

## SUBSEQUENT TREATMENT

We have seen that the immediate effect of quinine and other drugs is to allay the febrile symptoms and to cause the disappearance of parasites, but this condition of apparent cure was, sooner or later, followed by a relapse in the majority of cases. Two questions consequently arose:—

1. The first was, could the condition of apparent cure be *maintained* by continuing the quinine treatment, and if so, how should it be given?

2. The second was, were these cases in which the administration of quinine was continued for more or less long periods, and which showed no symptoms while taking quinine, really cured? Would they relapse or not, when treatment was stopped, just as they had done when the treatment had lasted only a few days, or would the number of relapses be now smaller

## QUESTION I.

The aspect of the problem that mainly occupied us was, whether if a certain total dose of quinine were given weekly, e.g., grains 30, 60, 90, it were better to administer the quantity on 6 days giving 5, 10, or 15 grains daily, or on two consecutive days only each week, giving 15, 30, or 45 grains daily.

This question was put to the test for a period of eight weeks in a series of cases for each total weekly dose of 30, 60, and 90 grains of quinine sulphate.

An accurate record was kept of the febrile relapses (non-parasitic) and of the parasitic relapses (febrile and afebrile), as determined by the temperature chart and daily blood examinations during the whole of the period.

In each series the record was in favour of the weekly administration of quinine in preference to the daily.

Thus, 30 grains is better administered in the form of two doses of 15 grains, than in the form of six doses of 5 grains.

The best result was obtained by the administration of grains 45 (three doses of grains 15), on each of two consecutive days weekly, this as above stated, giving a better result than grains 15 daily for six days.

An interrupted treatment of 30 grains on each of two consecutive days weekly, also suffices to keep the blood free from trophozoites and to prevent relapses in the majority of cases (while the treatment lasts).

In other words, in order to maintain a patient in a condition of freedom from relapses, an *interrupted* course of quinine is preferable to a *continuous* one.

So far as the actual result was concerned, an equally good one, or nearly so, was obtained in a different way, viz., by giving 15 grains of bihydrochloride intramuscularly on each of the first two days of treatment, and then Liquor arsenicalis m30 daily, with two periods of intermission for eight weeks (two weeks on, one week off, two weeks on, one week off, two weeks on).

The comparative figures for this and the previous interrupted quinine treatment are the following :—

	Quinine injections, two only, followed by Liq. arsenicalis m30 daily	Quinine sulphate Gr. 45 on two consecutive days weekly for 8 weeks
Percentage of parasitic febrile relapse cases per cases treated (average per week) ... ..	2.7	1.8
Percentage of all febrile (parasitic and non- parasitic) cases per cases treated (average per week ... ..	8.7	10.3
Number of cases treated... ..	33	74

What we have just considered is a method of maintaining freedom from relapses *while the treatment is in force*. We shall now consider a different question, viz. :—

#### QUESTION 2.

This question resolves itself into an enquiry as to whether by any course of treatment, short or long, a curative effect would be obtained, i.e., freedom from relapses after cessation of treatment, over an observation period of sixty days (or longer).

Many methods were tried, but in nearly all, when treatment was stopped, the number of relapses was large, and there is at present no method known which will cure all cases, even if the treatment lasts eight weeks.

Many methods of cure continue however, to be advocated, but they are not supported by trustworthy evidence, more especially in regard to an adequate observation period.

The following two treatments gave us the best results:—

Number of cases treated	Number of cases not relapsing but lost sight of before the expiration of 60 days	Number of cases relapsing in an observation period of 60 days	Relapses per cent.		Time of Year.
			min.	max.	
Liquor arsenicalis minims 30 daily with 1 or 2 periods of intermission with an injection of quinine bihydrochloride on each of the first two days only	32	—	4	12.5 12.5	End of treatment August:—1 case; September:—17 cases; October:—14 cases NOTE.—One additional case was not controlled by the treatment.
Novarsenobillon 0.9 grm. intravenously on the 1st, 8th, and 15th days with quinine bihydrochloride grs. 15 intramuscularly on the 1st and 2nd, 8th and 9th, and 16th days.	12	1	—	8.3 16.6	End of treatment December:—12 cases

It is worthy of note that a treatment which is 'good' whilst it lasts is not necessarily followed by a 'good' result when it has ceased. Thus the treatment noted above, viz., grains 45  $\times$  2 weekly for eight weeks, while 'excellent' while it lasted, was followed by 80 per cent. of relapses when the treatment had finished.

Whereas the arsenic treatment also a good one while it lasted, was followed by a 'good' result also when it had ceased.

**THE DISAPPEARANCE OF MALIGNANT TERTIAN GAMETES  
(CRESCENTS) UNDER QUININE TREATMENT**

1. With a dose of grains 30 or 45 daily, crescents do not persist in the blood in the majority of cases for more than three weeks. Whether they would disappear equally rapidly without quinine we did not determine.
2. Similarly with quinine sulphate grains 30 on each of two consecutive days weekly for five weeks, the crescents diminished from 50 per cent. in the first week to 6 per cent. in the fifth week of treatment.

## APPENDIX

The Titles, number of volume and date of Publication of the Studies in the Treatment of Malaria (I-XXXI) are given below :—

## ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY.

- I Intravenous Injections of Tartar Emetic. Vol. XI, p. 91. 1917.
- II Intramuscular Injections of Quinine Bihydrochloride in Simple Tertian Malaria. Vol. XI, p. 113. 1917.
- III Intravenous Injections of Quinine Bihydrochloride. Vol. XI, p. 149. 1917.
- IV Intramuscular Injections of Anylopsin and Trypsin in Simple Tertian Malaria. Vol. XI, p. 165. 1917.
- V Intramuscular Injections of Quinine Alkaloid in Simple Tertian Malaria. Vol. XI, p. 173. 1917.
- VI Oral Administration of Quinine for Two Consecutive Days only in Simple Tertian Malaria. Vol. XI, p. 283. 1918.
- VII Oral Administration of Quinine Sulphate daily over Prolonged Periods in Simple Tertian Malaria. Vol. XI, p. 309. 1918.
- VIII Oral Administration of Quinine Sulphate for Two Consecutive Days Weekly over Prolonged Periods in Simple Tertian Malaria. Vol. XI, p. 331. 1918.
- IX A Comparison of the Results of Interrupted and Continuous Quinine Treatment. Vol. XI, p. 359. 1918.
- X Oral Administration of Quinine Sulphate Grains 120 on Two Consecutive Days only in Simple Tertian. Vol. XI, p. 417. 1918.
- XI Oral Administration of Quinine Sulphate Grains 90 on Two Consecutive Days Weekly over a Period of Three Weeks in Simple Tertian Malaria. Vol. XI, p. 421. 1918.
- XII At what Time after Cessation of Quinine Treatment do Relapses occur in Simple Tertian Malaria? Vol. XI, p. 425. 1918.
- XIII Oral Administration of Quinine Sulphate Grains 90 on Two Consecutive Days only in Simple Tertian Malaria (Second Series). Vol. XII, p. 71. 1918.
- XIV Quinine Bihydrochloride Grains 30 intramuscularly, and Quinine Hydrochloride Grains 30 orally, Daily for 12 days, in Simple Tertian Malaria Vol. XII, p. 197. 1918.
- XV A Factor hitherto overlooked in the Estimation of the Curative Value of Treatments of Malaria. Vol. XII, p. 201. 1918.
- XVI Intravenous Injections of Novarsenobillon in Simple Tertian Malaria. Vol. XII, p. 211. 1918.
- XVII Oral Administration of Quinotoxin for two Consecutive Days only, in Simple Tertian Malaria. Vol. XII, p. 217. 1918.
- XVIII. A Comparison of the Value of Continuous and Interrupted Quinine Administration in Simple Tertian Malaria (Second Communication). Vol. XII, p. 303. 1919.
- XIX Intravenous Injections of Disodoluargol in Simple Tertian Malaria. Vol. XII, p. 339. 1919.
- XX Intramuscular Injections of Collosol Manganese in Simple Tertian Malaria. Vol. XII, p. 345. 1919.
- XXI Arsenic in Simple Tertian Malaria. Vol. XII, p. 371. 1919.
- XXII Intramuscular Injections of Quinine Bihydrochloride Grains 15 on each of two Consecutive Days only, in Malignant Tertian Malaria. Vol. XIII, p. 63. 1919.



- XXIII Oral Administration of Quinine Sulphate Grains 30 on each of two Consecutive Days weekly, over a Period of Five Weeks in Malignant Tertian Malaria. Vol. XIII, p. 69. 1919.
- XXIV The Disappearance of Crescents under Quinine Treatment. Vol. XIII, p. 73. 1919.
- XXV Arsenic in Malignant Tertian Malaria. Vol. XIII, p. 75. 1919.
- XXVI The Action of Arsenic and of Quinine on Quartan Malaria. Vol. XIII, p. 97. 1919.
- XXVII Intravenous Injections of Novarsenobillon and Intramuscular Injections of Quinine Bihydrochloride in Simple Tertian Malaria. Vol. XIII, p. 101. 1919.
- XXVIII Quinine Hydrochloride in Simple Tertian Malaria. Vol. XIII, p. 117. 1919.
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## NOTES ON AUSTRALIAN CESTODES

BY  
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No. VII.

In this paper, which is the last of the series, three new species and one new genus are described. Further information relating to the morphological characters of *Monopylidium macracanthum* and *Linstowia echidnae* are also included.

*Bothridium ornatum*, n. sp.

On several occasions specimens of this worm were obtained from Carpet Snakes (*Python spilotes* var. *variegatus*, Gray), taken in the Townsville district.

EXTERNAL ANATOMY.

The largest worm measured about 65 cm. in length, and the greatest breadth was 7 mm.

*Head.* The head measures about 4 mm. in breadth and 5 mm. in length. It consists of two cylindrical muscular tubes, one lying dorsally and the other ventrally. They are attached to each other throughout their whole length by a broad membrane. They are funnel-shaped and are open at both ends, the posterior opening being the smaller and directed inwardly (fig. 1).

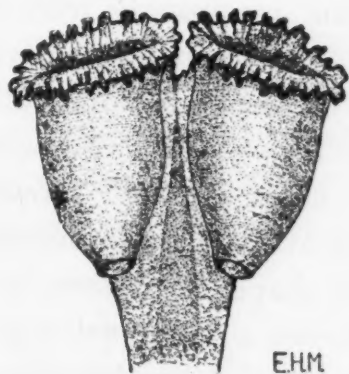


FIG. 1. *Bothridium ornatum*, n.sp. Head.  $\times 9$ .

Unlike *B. pithonis*, the anterior opening is surrounded by a very conspicuous fleshy frill.

*Segments.* These are very numerous, and are all broader than long. The lateral borders are imbricated.

#### INTERNAL ANATOMY.

No points of difference could be observed between this worm and *B. pithonis*, as described by Braun (1900).

#### DIAGNOSIS.

We have compared our specimens with the literature of all the known species of *Bothridium* and find that it differs from them in the characters of the head, viz., the possession of a fleshy frill round the anterior openings. It is proposed to name it *Bothridium ornatum*, n. sp.

Type specimens are in the Museum of the Liverpool School of Tropical Medicine.

#### *Monopylidium fieldingi*, sp. nov.

These cestodes were found in the intestine of several specimens of the Butcher bird (*Cracticus destructor*, Temm.), all of which were shot in the neighbourhood of Townsville, North Queensland.

#### EXTERNAL ANATOMY.

A complete specimen of the worm was not available for examination, so the total length cannot be given, but, from the appearance of several fragments taken together, and the rate of development, it is estimated that a complete adult would be over 50 mm. in length. The maximum breadth attained is 1.2 mm.

*Head.* The rostellum is strongly retracted in all the scolices available for study, with the result that anteriorly, it is in the shape of a truncated cone. The rostellum apparently invaginates when in this state, so that the tip is in the form of a saucer-shaped depression, around the edge of which is a double crown of alternating hooks (fig. 2). The hooks are about forty in number in each row and are of a definite rose-thorn shape when seen in profile; when viewed dorso-ventrally they present a Y-shaped appearance, the handle of which is long and the limbs of unequal length. They measure about  $22\mu$  in length (fig. 3).

The scolex reaches its maximum breadth (about 0.4 mm.) across the posterior borders of the suckers. These organs, when viewed in profile, are seen to stand out slightly from the surface. They are circular in shape, and measure about  $130\mu$  in diameter. They look outwards and slightly forwards, and are unarmed.

Immediately behind the suckers the scolex narrows slightly, and its termination is marked by a somewhat indefinite constriction (probably an artifact), which lies about 0.4 mm. from the anterior end. Immediately behind this constriction is an unsegmented portion about 0.4 mm. in length and 0.25 mm. in breadth. At this point, *i.e.*, about 0.8 mm. from the anterior extremity, the first traces of segmentation appear.

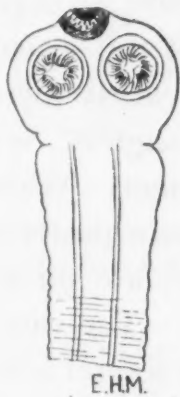


FIG. 2. *Monopylidium fieldingi*, n.sp.  
Head.  $\times 35$ .



FIG. 3. *Monopylidium fieldingi*, n.sp. Hook, highly magnified.

*Segments.* Segmentation soon becomes quite distinct, and the proglottides are seen to have their lateral borders curved, with the convexity outwards, and the postero-lateral angles projecting fairly widely. After about the fiftieth proglottis the length increases slightly more rapidly than the breadth, so that the proportion of breadth to length alters somewhat, but never to such an extent that the length becomes greater than the breadth.

Mature proglottides measure 0.17 mm. antero-posteriorly, 0.4 mm. across the anterior, and 0.47 mm. across the posterior border. The medullary portion at this stage is only 0.170 mm. in breadth.

#### INTERNAL ANATOMY.

The longitudinal muscle consists of relatively thick fibres, which are disposed in two layers, but, as the material was not in a good

enough state of preservation, and was also somewhat scanty, sections could not be cut, and therefore a detailed description of the musculature cannot be given.

*Nervous system.* The details of this system were not investigated.

*Excretory system.* The longitudinal excretory vessels lie at a considerable distance from the lateral borders, so that the medulla is correspondingly narrow. The ventral vessels are uniform in diameter throughout their whole length; they measure about  $20\mu$  in optical section. The narrower dorsal vessel lies directly above the ventral, and the ducts from the reproductive organs pass between them.

*Genitalia.* The genitalia develop slowly, so that there are about one hundred segments showing traces of the sexual organs before they become sufficiently developed to be clearly distinguished.

*Testes.* The testes are circular or slightly oval, and number about sixteen to twenty-one in each segment. When viewed dorso-ventrally they are seen to occupy the space posterior to the female glands, but on each side a few follicles are on a level with the vitellarium, or even with the ovary itself. The vasa efferentia unite into a vas deferens, which is thrown into several coils in front of the right lobe of the ovary. There is no external vesicula seminalis, and the vas deferens passes directly into the base of the cirrus pouch. The cirrus pouch is relatively long and narrow, its dimensions being  $130\mu$  in length and  $45\mu$  in breadth. Beginning mesial to the excretory vessels, it runs towards the right side in all cases, and very slightly posteriorly, and, passing between them, opens in a small atrium, which in turn opens on the right lateral border, about the junction of the anterior and middle thirds. The characters of the cirrus could not be made out (fig. 4).

*Receptaculum and vagina.* The vagina is a long straight tube which commences at the genital pore, immediately posterior to the opening of the cirrus. From here it runs transversely inwards, thus diverging more and more from the cirrus pouch as it goes; it passes dorsal to the right lobe of the ovary, dilating over the ovarian duct into a small but distinct receptaculum seminis.

*Ovary.* The ovary is centrally situated in the anterior half of the medulla. It is approximately bilaterally symmetrical and



consists of three lobes, two pointing laterally and a median lobe pointing anteriorly (fig. 4).

*Uterus.* The uterus develops as a uniform sac devoid of out-pocketings. It eventually fills the entire segment antero-posteriorly and extends laterally to the excretory canals.

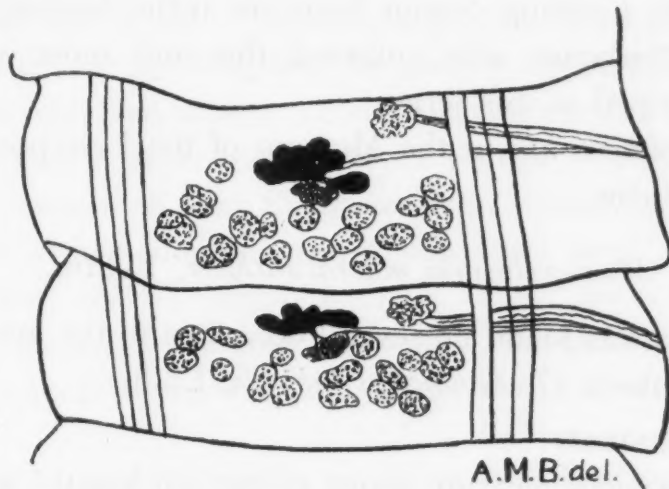


FIG. 4. *Monopylidium fieldingi*, n.sp. Ripe segment, showing genitalia.  $\times 69$ .

It is split up into capsules having a reticular form, each capsule containing up to about twelve eggs. Later, a separate capsule appears to be formed around each egg. The uterus was not fully

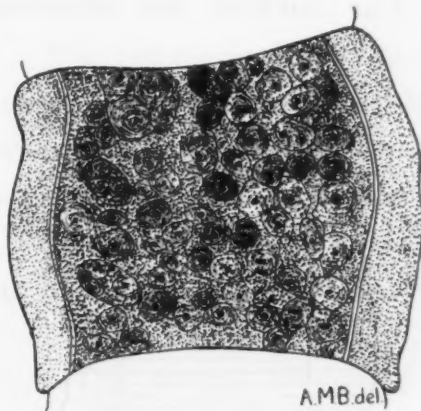


FIG. 5. *Monopylidium fieldingi*, n.sp. Gravid segment.  $\times 28$ .

matured in any specimen, therefore the nature and extent of these capsules could not be determined (fig. 5).

*Eggs.* No ripe eggs were seen.

## DIAGNOSIS.

As this worm possesses all the characters given by Fuhrmann (1899) in the diagnosis of *Monopylidium*, there is no doubt that it belongs to this genus.

As it disagrees with all known species of *Monopylidium*, it is consequently new and accordingly named *Monopylidium fieldingi* after Mr. J. W. Fielding, Senior Assistant at the Australian Institute of Tropical Medicine, who collected this and most of the other material described in this series.

Type specimens are in the Museum of the Liverpool School of Tropical Medicine.

*Monopylidium macracanthum*, Fuhrm.

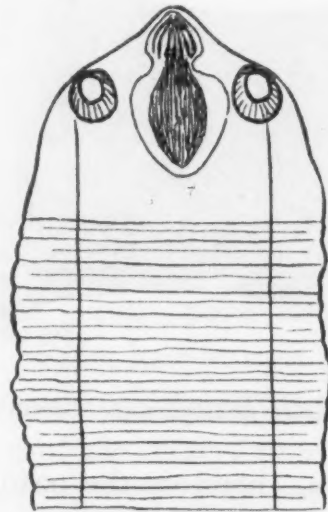
This worm was found on several occasions in the intestine of the spur-winged plover (*Lobivanellus lobatus*, Lath.).

## EXTERNAL ANATOMY.

Fixed specimens measure about 45 mm. in length, and 1.8 mm. in breadth, but there is apparently some shrinkage from fixation, so that these dimensions will have to be revised when fresh material is available.

There is no trace of a 'neck', the scolex passing directly into the segmented chain.

*Head.* The scolex is about 0.6 mm. in breadth, and is conical anteriorly (fig. 6). The suckers are relatively small, but well



E.H.M.

FIG. 6. *Monopylidium macracanthum*, Fuhr. Head.  $\times 35$ .

developed; they measure 0.13 mm. in diameter, and are situated on the scolex just where it begins to narrow anteriorly. They face forwards and slightly outwards. The tip of the scolex bears a rostellum, which was only seen in the retracted state in all the specimens available for examination.

*Rostellum.* The rostellum is a muscular organ, and completely fills the fossa into which it is contracted; this fossa measures about 0.47 mm. in depth and 0.24 mm. in greatest breadth, being oval in shape. The rostellum is seen to consist of two parts, a small anterior conical part and a larger oval posterior portion. The anterior part is distinctly marked off from the posterior by a neck-like constriction. It is armed with a double row of relatively large hooks, which number twenty-six (? twenty-eight). They are of two sizes, alternating, the larger measure  $145\mu$  and the smaller about  $110\mu$ . They have a long dorsal root and blade, and a short ventral root,  $145\mu$  (fig. 6).

The posterior portion of the rostellum is oval in optical section, and, in the contracted state, appears very muscular.

*Segments.* The dimensions of sexually mature segments are about 1 mm. across the anterior, and 1.2 mm. across the posterior borders, so that the postero-lateral angles are only slightly projecting; their length is 0.4 mm., giving a proportion of breadth to length of approximately three to one (fig. 7).

#### INTERNAL ANATOMY.

*Muscular system.* The cuticle is thickly studded with calcareous corpuscles, and the muscle layers are only thinly developed, but their exact disposition cannot be given as there was not sufficient material from which to cut sections.

*Nervous system.* This was not carefully investigated. It was noted, however, that a single nerve ran external to the excretory vessels.

*Excretory system.* The lateral excretory vessels are situated directly one above the other, the dorsal being the narrower. The ventral vessels are joined by a commissural channel, which runs across immediately posterior to the testes.

*Genitalia.* The reproductive organs can first be made out in about the tenth proglottis, and from here on they steadily become more distinct, until they reach maturity. There are about fifty

proglottides sexually mature as far as microscopic characters go, before the uterus becomes apparent.

The genital pores are circular, relatively large and irregularly alternating.

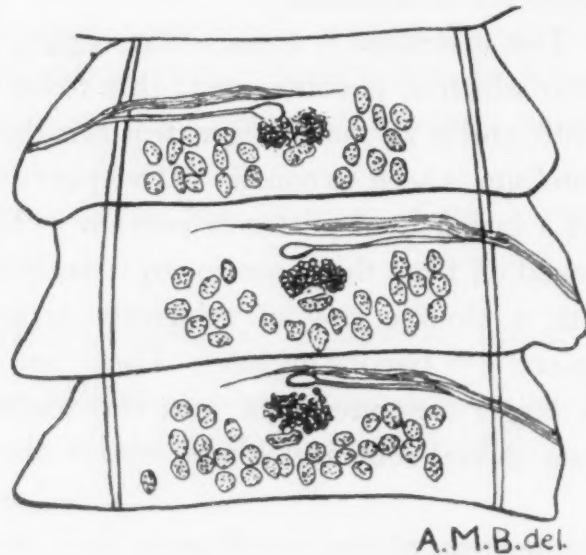


FIG. 7. *Monopylidium macracanthum*, Fuhr. Segments showing genitalia.  $\times 35$ .

*Testes.* The testes number twenty-three to thirty in each segment, and they occupy the usual dorsal position lying on each side of, and posterior to, the ovary when viewed from above (fig. 7).

*Vas deferens.* The various vasa efferentia pass forwards and unite in front of the ovary into a many-coiled vas deferens, which apparently fulfils the function of a vesicula seminalis, as there is no indication of this organ otherwise. These coils lie transversely on the pore side about the junction of the anterior and middle thirds of the segment, and run direct into the mesial end of the cirrus sac. The cirrus pouch is long and thin, and contains a few coils of vas deferens and the cirrus. In its course it runs laterally between the excretory vessels, and, passing slightly posteriorly, opens about the middle of the lateral border of the segment. The characters of the cirrus could not be clearly determined, as it was in all cases entirely within the pouch.

*Ovary.* The ovaries consist of two equal lobes placed one on each side of the mid-line, and each is composed of many subsidiary branches (fig. 7). The anterior border of the ovary is about the



level of the mid-transverse plane of the segment. The lobes each measure about  $80\mu$  in diameter. A duct runs inwards from each lobe and they unite in the mid-line to form the oviduct which joins the fertilisation canal running to the uterus. From this junction a duct passes directly backwards to the vitellarium, and it is surrounded for a little part of its length by the small compact shell gland.

*Receptaculum and vagina.* The vagina opens from the genital atrium, ventral and posterior to the male opening. It is a straight tube which at first follows the posterior border of the cirrus pouch as it passes mesially, but it soon leaves this and runs directly inwards towards the anterior border of the ovary, where it dilates in front of the lobe on the pore side, into a small receptaculum seminis; from the mesial end of the receptaculum a duct passes posteriorly to join with the two branches of the ovarian duct (one from each lobe), where they unite.

*Vitelline glands.* The vitellarium lies behind the ovary in the mid-line. It is a compact body, showing no trace of branching, but is somewhat indefinitely divided into right and left lobes, with the result that the whole organ is more or less kidney-shaped, with the 'hilum' facing forwards. A duct runs from its centre anteriorly, to join the fertilisation canal.

*Uterus and eggs.* The uterus is at first simple, and saccular, but later it splits up into capsules, each containing a single oncosphere. The eggs are circular or slightly oval, being about  $80\mu$  in diameter; the contained embryo measures about  $30\mu$ .

The male copulatory organs persist and are quite distinct even in fully gravid segments, long after all the other reproductive organs have entirely disappeared.

#### DIAGNOSIS.

The presence of a double crown of hooks, identical in size with those of *Monopylidium macracanthum*, Fuhrmann, together with the uterus split up into capsules each containing one oncosphere, leaves no room for doubt that this species is *M. macracanthum*, Fuhrmann. The only point in which it differs is in the number of hooks. Fuhrmann gives twenty-two, whereas in the present species at least twenty-six were seen clearly. As hooks easily become



detached, and further, as their number often varies, no importance can be placed on this difference.

Fuhrmann (1907) originally recorded this parasite from *Helodroncus octopus* in Africa and India, and as his description is somewhat meagre, it was thought desirable to amplify his account when making this new record of the worm in a fresh host and locality, viz., *Lobivanellus lobatus*, from North Queensland.

Type specimens of this cestode were placed in the Museum of the Liverpool School of Tropical Medicine.

*Linstowia echidnae*, Thompson (1893).

D'Arcy Thompson (1893) described a cestode from the Echidna from Australia. In his brief description he mentions that the worms were very contracted.

We have a large collection of immature worms from the same host which, as far as can be ascertained, are the same species. Our material, however, is not so strongly contracted as that of Thompson, and accordingly the condition of the scolex in particular is somewhat different.

As Thompson's description is rather incomplete, the following additional particulars are given.

*Head.* The anterior surface of the scolex is quite devoid of a rostellum, in fact in some cases it has a slight central depression.

The dimensions of the rounded scolex differ slightly in different specimens, varying between 0.76 mm. and 0.58 mm. in breadth. The maximum diameter is just posterior to the suckers.

*Suckers.* The four suckers are placed well forward on the scolex; they are well developed, circular organs, lying flat on the surface, and their openings look outwards and slightly forwards (fig. 8).

*Segments.* At first the proglottides are almost rectangular in shape, broader than long, with no projection of the postero-lateral angles; but as development advances the posterior angles come to project somewhat, with the result that the anterior borders of the segments are shorter than the posterior. The dimensions of the most fully developed segments available for study are 1.6 mm. across the posterior borders, and about 1.35 mm. across the anterior, with a length of about 0.45 mm., being approximately a proportion of breadth to length of three to one. The posterior border is slightly

curved, with the convexity backwards, and to some extent it overlaps the succeeding segment. The cuticle of the worm is thrown into several slightly marked longitudinal folds, which on the



FIG. 8. *Linstowia ecbidnae* (Thompson). Head and anterior segments.  $\times 12$ .

posterior free borders of the segments give an appearance of scalloping (figs. 8 and 10).

#### INTERNAL ANATOMY.

*Muscular system.* Transverse sections show a relatively thick cuticle and cortical parenchyma, and the longitudinal muscle is disposed in two layers, completely encircling the segment, the outer layer being slightly the thicker of the two (fig. 9).

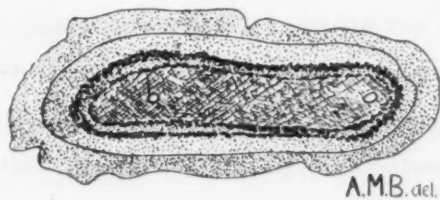


FIG. 9. *Linstowia ecbidnae* (Thompson). Transverse section showing musculature.  $\times 35$ .

*Nervous system.* This system was not investigated.

*Excretory system.* The dorsal excretory vessel is narrower than the ventral, and lies to the outer side of the latter. They both pass dorsal to the ducts of the male and female organs.

*Genitalia.* The genital pores cannot be made out, as in no instance is development complete enough to show them, but from the direction of the immature sex ducts they would probably open about the centre of the lateral border; they are irregularly alternating, there being, as a rule, three or four on one side followed by about the same number on the other side. The reproductive organs are single in each proglottis (fig. 10).

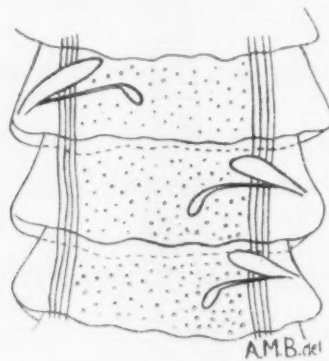


FIG. 10. *Linstowia echidnae* (Thompson). Segments showing cirrus pouch and vagina.  $\times 35$ .

*Testes.* Owing to the immature condition of the worms, only traces of the testes can be distinguished. They are numerous and are scattered dorsally across the whole width of the medulla.

*Vas deferens.* No details of this organ can be made out, but it is seen entering the mesial end of the developing cirrus pouch. At the most advanced stage of development observed, the cirrus pouch is represented by a relatively long, straight, tubular structure, which runs inwards and slightly forwards from opposite the centre of the lateral border, so that it lies across the antero-lateral angle of the segment on the side on which it will eventually open.

*Ovary.* This organ lies slightly to the pore side of the median line, and about midway between the anterior and posterior borders of the segment. No details of its structure can be given because it is quite immature.

*Receptaculum and vagina.* The vagina is seen as a straight tube, running inwards along the posterior border of the cirrus pouch, which it leaves about its centre, and running directly inwards, ends in a small expansion, evidently the beginnings of a receptaculum seminis, around which the developing female genitalia can be seen.

On account of the undeveloped condition of the worms, no particulars of the vitelline glands, shell glands, uterus or eggs can be given.

*Paramoniezia suis*, n.g., n. sp.

One specimen was obtained from the intestine of a wild pig (*Sus scrofula*), near Townsville, North Queensland.

EXTERNAL ANATOMY.

The worm is lancet-shaped and measures about 12 cms. in length and 10 mm. maximum breadth.

*Head.* The head is very small and measures only about  $300\mu$  in breadth. It is unarmed and there is no rostellum. The suckers are extremely small; they were too shrunken to give accurate dimensions. There is no neck.

*Segments.* These are always broader than long and their free edges are imbricated. A typical mature segment measures  $200\mu$  in length and 9 mm. breadth. The genital pores are double.

INTERNAL ANATOMY.

*Muscular system.* The longitudinal muscle fibres are arranged in a single layer, composed of numerous bundles measuring about  $60\mu$  thick. External to this is a thin layer of circular fibres. A few dorso-ventral fibres also occur.

*Nervous system.* This system could not be investigated because even the main lateral nerve could only be seen with difficulty.

*Excretory system.* The ventral excretory vessel is very large, and in most transverse sections it appears to occupy the whole of the lateral dorso-ventral space. The diameter of the tube is about  $150\mu$ .

The large size of this vessel, and the numerous branches to which it gives rise, made it difficult to determine whether a dorsal vessel was present or not; but careful examination led us to the conclusion that a dorsal vessel was absent.

*Genitalia.*

*Testes.* These are very numerous (at least three hundred). They extend on each side almost to the lateral extremity of the segment and are not grouped round the ovary, but extend right



across the segment. Each testis measures about  $65\mu$  by  $45\mu$ . Antero-posteriorly they lie in four or five rows, and dorso-ventrally in from one to three layers.

*Vas deferens.* The vas deferens on each side runs dorsal to the ventral excretory vessels. The cirrus pouch is tubular and lies lateral to the water vessel. Its median portion contains an internal vesicula seminalis. No external vesicula seminalis was seen. The cirrus is unarmed.

*Ovary.* This organ is paired in each segment, and is of the usual *Cittotaenia* or *Moniezia* type.

*Receptaculum and vagina.* From the pore the vagina runs inwards, having at first a diameter of about  $60\mu$ ; it expands immediately internal to the excretory vessels into a large transversely elongated muscular sac (the receptaculum seminis) measuring about  $650\mu$  in length and  $150\mu$  breadth. Its median extremity, which lies close to the ovary, is continued as a short coiled narrow tube to the fertilization canal.

A most important point is the fact that whilst the vagina is always ventral to the cirrus pouch on right side, it may be either dorsal or ventral on the opposite side.

*Uterus.* This is first apparent as a cell-string running across the segment. It develops into a tube, devoid of outgrowths, and extends on each side to the extreme edge of the segment.

*Eggs.* The ripe egg has a diameter of  $45\mu$ ; the outer shell has a double contour. The hexacanth embryo measures about  $24\mu$ , and a pyriform apparatus is entirely absent. Between the embryo and the shell a small quantity of yolk can be seen.

#### DIAGNOSIS.

This worm obviously belongs to the family *Anoplocephalidae*, Fuhrm., 1907, and the sub-family *Anoplocephalinae*, Blanchard, 1891. The only two genera within this sub-family possessing double genital pores and a single cirrus pouch on each side are *Cittotaenia*, Rheim., 1881, and *Moniezia*, Blanchard, 1891, and these differ from each other in one particular only, viz., in *Cittotaenia* the vagina is ventral to the cirrus pouch on both sides, whilst in *Moniezia* the vagina is ventral to the cirrus pouch on the right side and dorsal on the left. In the present species the relationship of the cirrus to vagina is variable, the vagina being



sometimes dorsal and sometimes ventral to the cirrus on the left side, in the same strobila.

It is necessary, therefore, to erect a new genus for this species, which we have named *Paramoniezia suis*, n.g., n. sp.

The characters of the new genus are as follows:—*Paramoniezia*. With the characters of the genus *Moniezia*, except that on the left side the cirrus is sometimes dorsal and sometimes ventral to the vagina.

Type specimens are in the Museum of the Liverpool School of Tropical Medicine.

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# A NOTE ON *OPHIOTAENIA PUNICA* (CHOLODOVSKI, 1908), LA RUE, 1911

BY  
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AND  
S. ADLER

(Received for publication 3 July, 1923)

Two specimens, both gravid, were obtained from the intestine of *Causus rhombeatus*, in Freetown, Sierra Leone.

## EXTERNAL ANATOMY.

The larger complete specimen measured 9 cms. in length, and the maximum breadth was 3.3 mms.

*Head.* The head is almost square and measures 1.5 mm. broad; it is unarmed.

*Suckers.* The four suckers have a diameter of 0.67 mm. The neck is 0.7 mm. long.

The worm is made up of about one hundred and forty segments. The first proglottides are broader than long; they gradually lengthen towards the posterior, the last proglottis being 4 mm. long and 2 mm. broad.

The genital pores are irregularly alternate, and open at the middle of the lateral border.

## INTERNAL ANATOMY.

*Musculature.* The musculature consists of a series of (1) small subcuticular fibres, situated immediately beneath the cuticle, (2) a double layer of longitudinal muscles which are not strongly developed, (3) a few diagonal fibres, and (4) circular fibres which are very scanty.

*Excretory system.* There are two water vessels on each side, the ventral vessel being much larger than the dorsal vessel.

*Nervous system.* A single nerve is present on each side, lying lateral to the water vessels. The parenchyma is strongly developed.

*Genitalia.* The testes are confined to the lateral fields in front of the ovary, and median to the vitellaria. There are from one hundred and seventy to two hundred and thirty in each segment; they are oval in shape, their long axes being horizontal (fig. 1).

*Vas deferens.* The cirrus pouch first becomes evident about 15 mm. behind the head; it lies either anterior or posterior to the vagina and extends beyond the vitellaria, being up to  $670\mu$  in length (fig. 1).

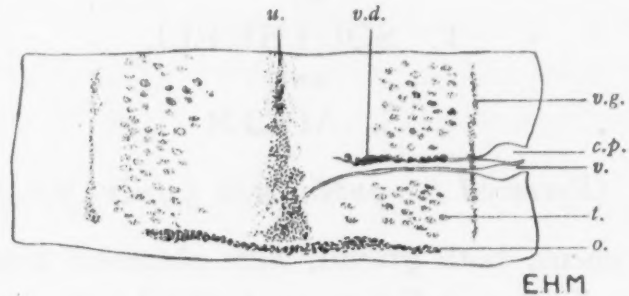


FIG. 1. *Ophiotaenia punica*. A ripe segment, shewing genitalia. *v.g.*—Vitelline glands. *c.p.*—Cirrus pouch. *v.*—vagina. *l.*—testes. *o.*—Ovary. *v.d.*—Vas deferens. *u.*—Uterus.  $\times 35$ .

The cirrus is spiny and is continuous with an internal seminal vesicle, which latter occupies about two-thirds of the cirrus pouch. The vas deferens lying outside the pouch is coiled.

*Ovary.* The ovary is long and narrow and is not bilobed (fig. 1); it is situated posteriorly.

*Vagina.* The vagina lies either anterior or posterior to the cirrus pouch. It runs almost straight towards the middle of the segment, and then turns posteriorly (fig. 1).

*Vitellaria.* The vitellaria are lateral, and consist of small acini measuring about  $30\mu$  to  $36\mu$  in diameter (fig. 1).

*Uterus.* The uterus is a straight tube running antero-posteriorly; in mature segments it has from eight to twelve lateral pouches on each side. There is a small shell gland situated immediately behind the middle of the ovary (fig. 2). In transverse

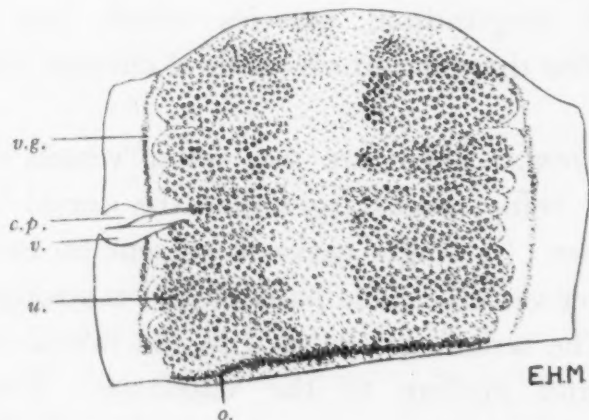


FIG. 2. *Ophiotaenia punica*. A segment shewing gravid uterus. *v.g.*—Vitelline glands. *c.p.*—Cirrus pouch. *v.*—Vagina. *u.*—Uterus. *o.*—Ovary.  $\times 35$ .

sections of segments in which the uterus was gravid no uterine pores were seen.

*Eggs.* The eggs are  $30\mu$  in diameter, and in appearance resemble the eggs of *Hymenolepis nana*. The oncosphere is from  $13\mu$  to  $15\mu$  in diameter. The embryophore has a thickness of about  $3\mu$  (fig. 3).



FIG. 3. *Ophiotaenia punica*. Egg.  $\times 733$ .

*Diagnosis.* *Ophiotaenia punica* was first found in a dog in Tunis by Cholodovski (1908), but, owing to its morphological characters, Hall, Ransom and La Rue thought the true host was a snake. They presumed that the dog had eaten a snake. Southwell (1922) recorded this parasite from *Paradoxurus hermaphroditicus* (Malayan palm civet) in Calcutta.

This is the first definite record of *Ophiotaenia punica* from a snake.

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## A PYRRHOCORID BUG CAPABLE OF BITING MAN

BY

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So far as I am aware, the following notes contain the first record of the fact that a bug of the family *Pyrrhocoridae* has been found to bite man. The observation was made at Freetown, Sierra Leone, but so near to the time of my departure that little opportunity of carrying out experimental work on the subject was available.

The natural order Rhynchota or Hemiptera is divided into the sub-orders Heteroptera and Homoptera; the Heteroptera into Gymnocerata and Cryptocerata; the Gymnocerata into several families, of which the family Pyrrhocoridae is one; in a sub-family of this—the Pyrrhocorinae—occur many genera, to one of which, namely, *Dysdercus*, belongs the insect with which I am here dealing. Mr. Lang, of the British Museum, has kindly identified the species for me as *D. supersticiosus*, F.

*Dysdercus supersticiosus* was observed by me at Freetown, in 1921, specimens being found even indoors in the laboratory. At that time I made a few experiments in order to see if the insects would bite; the bugs were placed singly in wide test-tubes and applied to the arm, but they showed no inclination to bite or even stay on the skin, making, on the contrary, efforts to escape by climbing up the test-tubes; it was concluded, rather prematurely as it now appears, that they were entirely non-biting in so far as human beings are concerned. They were observed in numbers on the ground, especially in the vicinity of a silk cotton tree, *Eriodendron anfractuosum*, situated about a hundred yards from the laboratory. It was observed that they appear to see very well, as they are extremely sensitive to any movement made in their vicinity; also that they make off instantly and move away with

great rapidity when disturbed. This year, in April, the silk cotton tree pods were opening on the tree and it happened that on one or two days the wind drove the cotton along, in the direction of the ground on which the laboratory stands. In the flocculi of silk cotton which were wafted on to the ground were seen numerous small red bugs, which in most cases extricated themselves quickly and ran about actively. Occasionally a silk cotton seed was carried along with the floating fragments of fibre, and it was not unusual to see on the ground a seed covered with bugs, some of them with their beaks inserted into it and others trying to pierce it, the seed being pulled in all directions during the process. When the bugs grew larger some were observed to develop cannibal habits, more especially when placed together in test-tubes with no food.

On the 24th of April, 1923, while sitting out in front of the laboratory just after sunset I experienced a sharp bite on the front of the ankle. On looking to see what was biting I moved slightly and could observe no mosquito or other biting insect, but saw a red bug moving rapidly off my sock. It was not possible to be sure that the definitely painful bite was caused by the bug; the reaction was a small itching swelling which had disappeared in twenty-four hours. Two days later, on the 26th April, at the same place and time, a bite was again felt on the same ankle. Leaning forward carefully I saw a red bug biting busily through my thick black sock. The bug's body, as its beak went deeper and deeper into the skin, assumed an attitude which was nearly vertical; considerable irritation was felt at intervals during the time the biting was going on. The process was timed and had lasted nearly four minutes when the bug was disturbed by a large black ant, which, in passing rapidly across the ankle, collided with the bug. The latter instantly made off and was escaping when it was captured in a test-tube which had been kept at hand since the previous observation, so as to be ready should the opportunity arise again. The reaction was on this occasion also only local, but was much more definite. Itching and irritation were felt, and in an hour's time a circular swelling of the size of a sixpenny piece had developed, well raised in the centre. This remained and in two days was rather hard, but it went away gradually in about five days from the time of the bite. The bug was examined and found to be a last larval stage:

the dissection of it did not reveal the presence of blood in the alimentary canal, nor were flagellates found; but the dissection had to be carried out with the light of an oil lamp, which was unsatisfactory.

*Laboratory experiments.* Several experiments were carried out by placing single bugs in large test-tubes on the human skin, but in no case was biting observed, the bugs being only anxious to escape by climbing up the tubes. Too much stress should not be laid upon the negative results of these experiments, in view of the fact that bugs which had been seen attacking the seed of the silk cotton tree on the ground made no attempt to attack the seeds when placed with them in tubes similar to those used for the skin experiments. Ballou (1906) made a comparable observation. He says: 'Although the cotton stainers are known to feed on the ripe cotton seed about the gin-houses, they would not do this in the laboratory, nor would they feed on the seeds of the silk cotton.' It is probable that on account of the timidity of the bug, special methods of experimentation in the laboratory must be devised.

Success, however, attended an experiment which was carried out in England. Two bugs were placed in the toe of a black sock, which was then drawn half on to the foot. After some minutes a bite was felt on the dorsum of the foot, but in the attempt carefully to remove the sock the bug was disturbed and ceased biting; the reaction was an itching sensation with subsequent local swelling, and for a few days a red circular area on the dorsum of the foot was observed. Later the whole leg became swollen and oedematous, and intense itching occurred; there was no pain, however, and no enlargement of glands and no temperature. Parasites were not found in the blood, but there was eosinophilia reaching 50 per cent. In three weeks the swelling began to go down, and in a month was gone.

*The bionomics of Dysdercus.* This genus of bugs is best known from its association with cotton in most parts of the world, and it contains the majority of insects classed as cotton stainers. Maxwell-Lefroy gives a detailed account of the morphology and bionomics of *D. cingulatus* which occurs in India, as well as notes on other species; Ballou (1906) enumerates a large number of species which are found in the West Indies, with observations on the various stages

of the life-history in different species. Egg-laying commences within a few days after copulation is over, the eggs being laid to the number of up to a hundred in various sites, on the ground, under leaves, in the open bolls; Peacock, in *D. superstitiosus* in captivity, found egg clusters from twenty to one hundred and twenty; the egg-stage lasts about a week, and the larva which emerges undergoes during its growth five ecdyses. The adult is distinguished from the larva in most cases, according to Butler (1923), by the acquisition of wings; increase of number of joints in tarsi, and sometimes in antennae; transference of openings of scent glands from dorsal to ventral side; full development of sexual organs. The time taken for growth from the time the egg is laid till the adult bug stage is reached is forty-nine to eighty-six days for *D. cingulatus*, in India.

*Food Plants.* The chief food plants of these bugs are the following:—The cotton *Gossypium* sp.; the silk cotton, *Eriodendron anfractuosum*; the Okra or Bhindi, *Hibiscus esculentus*; the musk mallow, *Hibiscus abelmoschus*, and other plants of the Malvaceae.

*Other food.* *Dysdercus superstitiosus* was observed by me at Freetown feeding in large clusters on the carcase of a frog. Mansfield-Aders (1919-20), in his account of insects injurious to economic crops in the Protectorate of Zanzibar, states that he has on many occasions seen *Dysdercus fasciatus* feeding with avidity on fresh mammalian carcasses, skins, and skulls. Of *D. superstitiosus* in Zanzibar, he says that it is by no means a common species on cotton, but that the silk cotton is commonly attacked by it.

Lamborn (1914-15) says of Southern Nigeria, 'During the dry season the Pyrrhocorid bug, *Dysdercus superstitiosus*, F., was found in some numbers . . . and at this time they appeared to be able to thrive on almost any food, whether of animal or vegetable origin, for eight or ten were noticed feeding on a dead and sun-dried lizard and a batch of young nymphs was found on sheep's excreta.'

Peacock (1913-14) gives a coloured plate of the stages of *D. superstitiosus*. He records the observation which he made of a number of young stainers about three weeks old sucking a dead snail.

*Seasonal occurrence.* Lefroy gives the following account of the



sequence of breeding and feeding habits of *D. cingulatus* at Pusa :—

April-May—Extensive breeding on Simul (silk cotton).

June-July—Feeding miscellaneous Bhindi, Hibiscus, etc.

August-November—Breeding in cotton.

'In most parts of India breeding is of necessity confined either to the cotton season, to the season when Bhindi is in pod, or to the season when the Simul is in bearing.' These observations are of interest with regard to the effect of season and the presence of particular food plants on the numbers and vitality of a single species.

*Distribution of Species.* Of equal interest are the observations made by Ballou on the distribution of different species in the West Indies. One species may extend for a distance and then, apparently without any change of environment, stop and give place to a different species. This sudden demarcation of the limits of a species is most noticeable in the case of *D. ruficollis*, and it is stated 'in many instances only one locality is known for each species, and most of the others occur only in a few adjoining countries or islands.' The distributions of *D. andreae* and *D. delauneyi* give good examples of localization. It is noted by Lefroy that *D. evanescens*, Dist., is recorded from Sikkim, the Khasi and Garo hills, Burma, from the Bor Ghat, Bombay, also from Chapra.

*Modification of feeding habits.* Butler (1923) considers the possibility of rapid change of food habit among bugs. He refers to two species of Capsidae which are even now gradually establishing, or indeed have established, themselves in orchards, viz., *Plesiocoris rugicollis* and *Orthotylus marginalis*; the natural food plants of these insects are various species of *Salix*, 'and the attack upon orchards indicates a startling change of taste brought about by the temptation of well-nurtured plantations of apple trees in their neighbourhood.' In captivity Ballou fed stainers on cotton seed, portions of unripe cotton bolls, bits of sugar cane and pieces of banana.

*Duration of Life.* In the insectary *D. cingulatus* was kept by Lefroy for four months; life was long when conditions were not favourable, i.e., little food. I may observe here that a few specimens of *D. superstitiosus*, last larval instar and adult, which received no

food and which were kept at ordinary temperature, survived the voyage from Sierra Leone to England and lived for a week after arrival.

*Bacterial parasites of Dysdercus spp.* De Charmoy (1921) says that in Mauritius *Dysdercus* is known to transmit several bacterial diseases, and that it is the vector of an internal disease of the bolls similar to that described by Nowell and others in the West Indies.

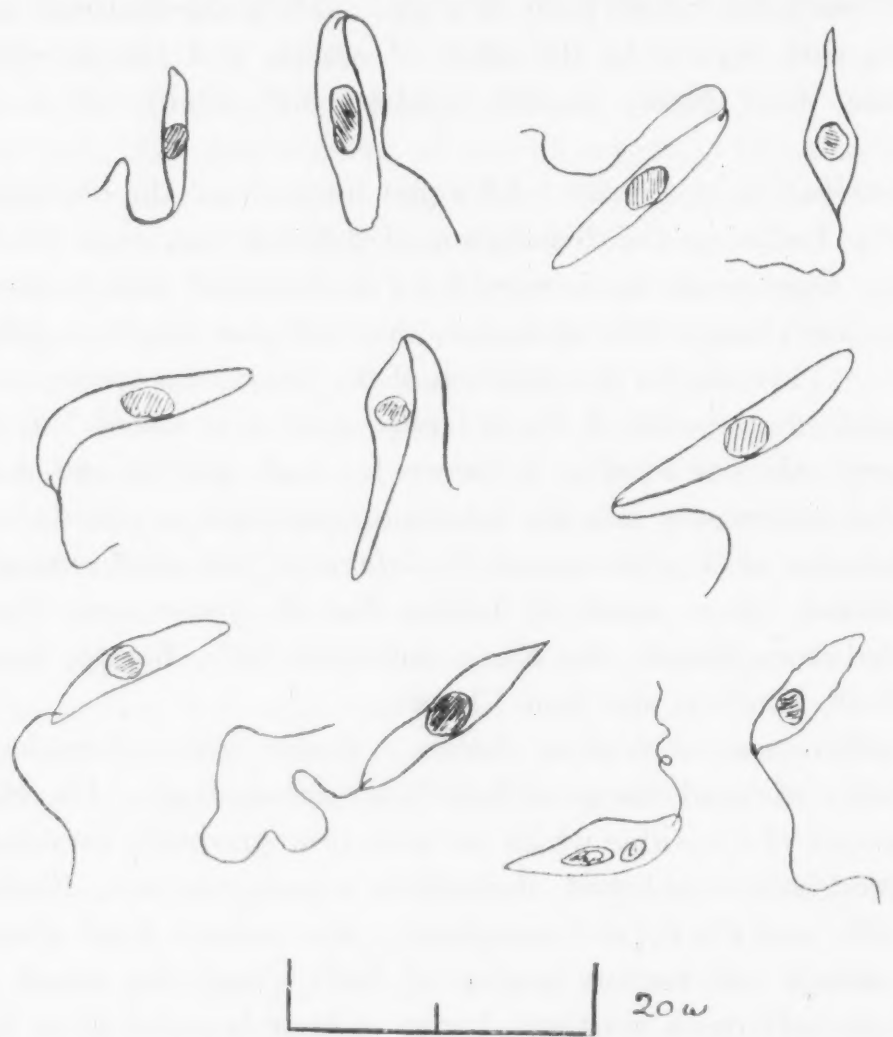


FIG. 1. Flagellate parasite of *Dysdercus supersticiosus*, F.

*Flagellate parasite of Dysdercus supersticiosus*, F. The bugs were found by me to be infected with a herpetomonas; it was present in various portions of the alimentary canal, and was recovered once from the coelomic fluid by cutting off the antenna near its base and examining the fluid which exuded; it was not found in the salivary glands, but as will be seen from the table subjoined, the number of dissections was limited:—

Flagellates (Herpetomonas) present in *Dysdercus supersticiosus* F.

Dissected	Infected	Rectum	Hind gut	Mid gut	Salivary glands	Coelomic Fluid
14	9	4	7	2	0	1

The occurrence of flagellates in Hemiptera-Heteroptera is well known. Patton and Cragg (1913) refer to the fact that tea, coffee and garden produce of all kinds are attacked by various species of bugs. They mention the family Lygaeidae, of which several species are infected with flagellates; *Oxycarenus laetus*, which is common on the cotton plant in Madras, is nearly always infected with a species of *Herpetomonas*; *Lygaeus pandarus* (*militaris*), which is common on the milk plant, *Calotropis gigantea*, is also infected with a flagellate of the same kind, *Herpetomonas lygaei*, which very closely resembles the parasite of Kala azar; *Lygaeus hospes* is infected with the same parasite. Of *H. lygaei*, Patton, the authors say that it is indistinguishable in its pre- and post-flagellate stages from the parasite of Kala azar as seen in man. The observations made by me on the flagellates of *Dysdercus supersticiosus* bear out these statements as regards the appearance of the *Herpetomonas* found, in the flagellate stage.

It is of interest to recall the discovery by Lafont (1909) of *Herpetomonas davidi* in the latex of *Euphorbia pilulifera* and to the presence of this flagellate in a species of Nysius. Miss Robertson has recorded a herpetomonas from the alimentary tract of *Dysdercus castatus*, the red cotton bug of Uganda; only a few infected specimens were examined. A still more important observation of Miss Robertson is in connection with the species *Leptoglossus membranaceus*, of the family Coreidae in Uganda; she found in its alimentary tract a herpetomonas; the parasite was found very frequently to invade the salivary glands. Miss Robertson considers notable 'the independent development in a sucking insect of all the factors requisite for the transmission of a flagellate, parasitic in the intestine by way of the mouth-parts of the insect host.'

The significance of the observation which I have made on the biting capability of *Dysdercus supersticiosus*, F., is evident from a

consideration of the foregoing facts. The work of Laveran and Franchini (1913) and of Fantham and Porter (1915) went to prove that insect herpetomonas may, when injected into animals, produce effects analogous to those occurring in Kala azar, the flagellates resembling cultural forms of *Leishmania donovani*, giving rise to the non-flagellated rounded forms. The partial development in the bed bug obtained by Patton of Kala azar parasites by feeding experiments is important, and especially so in view of the non-success which has hitherto attended all attempts to find an insect vector of the parasite of this disease.

Roubaud and Franchini (1922) obtained in mice infection with *Leishmania* forms of parasite by allowing fleas having a natural infection to breed in their box. The infection, which proves fatal to the mice, was conveyed to fresh mice by means of subcutaneous injection of ground-up tissues. These workers also obtained a similar result in mice by injecting into them the faeces of fleas.

Although there is, as yet, no evidence that *Dysdercus supersticiosus*, F., is capable of removing blood from man, there is ample evidence that in biting it is capable of injecting an irritating substance under the skin. This irritating substance can on analogy be none other than the salivary fluid; it is clear, therefore, that all the conditions for transference of a parasite to man are provided if salivary infection is present. Dr. P. A. Maplestone reports that he has found infection of the salivary glands.

There is a hypothesis put forward by Stephens (1915) to explain the lack of success of infecting arthropods from Kala azar cases. On this hypothesis—the hemi-cyclic hypothesis—it is possible that a biting arthropod may infect man by its bite; the parasite injected into man grows and multiplies in the tissues but does not enter the peripheral blood in sufficient numbers to cause an infection in the alimentary tract of a fresh arthropod when biting; this is tantamount to saying that the parasite which gets into the arthropod from some other source than man reaches in man a *cul de sac* from which it cannot escape. The hemi-cyclic hypothesis, however capable it might be of explaining transmission of disease by the bites of insects which were yet not capable of sucking blood, need hardly be considered here, as we do not know what are the actual capabilities of such bugs in general in this respect.



It may be noted that whereas Kala azar has a very limited distribution, the bed bug, in which early development has been observed, is world wide in its range. If an insect transmitter of Kala azar is to be found, it is probable that it will be more restricted in its distribution than is Cimex. Their localized distribution, their seasonal dependence on certain forms of plant food and their evident adaptability, point to bugs of the Pyrrhocoridae and similar families as objects of study. I believe my observations and experiments indicate the necessity for an exhaustive investigation of all such forms, not only in countries where Kala azar abounds but also in countries in which Tropical Sore occurs.

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## TYPHUS FEVER IN GREEK REFUGEES

BY

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During the last six months my duties in Greece have brought me into contact with about one thousand two hundred cases of typhus fever. From the nature of my work I saw the disease mainly from the standpoints of the medical administrator and the sanitary officer endeavouring to stamp it out in the areas allotted to the British Red Cross Society.

*Epidemiology.* The Greek refugees primarily brought the disease with them from Asia Minor and the Near East. With us the epidemic began at the end of January, 1923, gradually rose in number and severity of attacks, reached a maximum about the first fortnight in March, remained stationary a month or so and is now (June 22nd) dying out.

It is noteworthy that in the areas in which the refugees have been widely dispersed on the land there has been no typhus, or it has not gained a foothold when introduced. The brunt of the epidemic has been borne by the larger and medium-sized towns, the smaller either escaping, or being only slightly invaded. The city of Athens and its environs, Salonica, Patras, Corfu, and many other large towns, have suffered severely from the disease, whereas whole rural districts in Macedonia and Western Thrace have not been affected at all.

The housing conditions of the refugees, the hardships they have experienced, especially the insufficient food and exposure, have considerably reduced their stamina and vitality, and lowered their resistance to disease.

The outbreak attained its maximum of intensity in the mid-winter. In the colder weather the people are more crowded together, they wear thicker clothes which foster lice, and they bathe less frequently. Throughout the winter the refugees were packed with the ordinary civil population in the waiting-rooms of railway stations

and in other places; contact causes were therefore more frequent. In some small towns the number of refugees alone is equal to the former population; in others the refugees out-number the permanent inhabitants by two, or even three, to one. In the Landgada Valley (near Salonica) overcrowding has reached its possible limits. The ordinary population of the village of Landgada is five hundred; during the last six months one thousand six hundred refugees have been added to it. In one small refugee room, 9 ft. by 13 ft. as regards floor space and 5 ft. 6 ins. to the roof, I found four adult women and four children from 7 to 13 years old living; all the husbands were prisoners with the Turks; this was one of many similar overcrowded rooms above cowsheds. One could multiply instances of this sort; but overcrowding is only one of the many economic factors that enter into the epidemiology of typhus.

What has been happening is that infected refugees are necessarily brought into intimate contact with their non-infected comrades, all of whom were at one time (and to some extent are still) lice ridden. Infected lice are transferred to uninfected refugees. In the overcrowding that has arisen in nearly all the larger towns of Southern Greece, lousy infected refugees have also been brought into contact with the people of the permanent community, and the disease has thus spread to them. On several occasions I have seen, in organised refugee camps, typhus fever cases living with the uninfected and sharing the common bed, which is practically always the floor of the room or block they inhabit. The same takes place in railway stations where verminous typhus refugees infect the ordinary passengers in the waiting-rooms. Other hardships exist. A very large percentage of the refugees get only a bare subsistence allowance of food. There are districts in which the men who can work get no food dole and the out-of-work women only about six to eight ounces of bread or flour a day. For some months few refugees had a change of clothing; undergarments were especially scarce. With only one suit of clothes the practical difficulties of delousing are obvious; it is put through the steam disinfecter and dried while the refugee is having his bath. It is necessary to emphasise that the sanitary condition under which the refugees lived for months after their coming to Greece was very bad; it has been greatly ameliorated.

*Mode of Transmission.* The transmission of typhus by body and clothes lice may be accepted as proved; the infected human louse is the intermediary in the transmission of typhus from typhus cases to the healthy—ordinarily there is no direct communication of the disease from man to man. If it were possible to exterminate human lice in an infected area, the disease would cease. Further, if we could rid all typhus cases of lice the disease would come to an end. All our radical preventive measures are based on these facts. Somehow it has got abroad that it is only the body-louse that acts as a carrier. This is not the case; the exculpation of *P. capitis* is a dangerous and pernicious theory to inculcate.

*Period of Incubation.* A generation or so ago, twelve days used to be given as the period of incubation. All recent experience of the disease in Russia and Greece shows this to be correct. It has been proved by the inoculation of human blood experimentally; inoculation of monkeys with infective human blood has likewise demonstrated it.

Most cases are admitted into infectious diseases hospitals on the fifth day of the disease, the next most frequent is the sixth day. This late admission arises from several causes—antipathy of the refugees to typhus hospitals, their ignorance as regards the nature of the disease, inability to obtain medical advice, overwork of the doctors, etc. In all but a very small proportion of cases the eruption is out by the time the patients reach the hospital.

*Symptoms.* In the vast majority of cases there are prodromata in the form of ill-defined malaise with vague symptoms for two or three or even four days before more severe indications arise. The real onset is well-defined. In typical cases the patient knows the day, often the hour, when he first felt genuinely ill and had to go to bed. The face is then somewhat flushed, the conjunctiva injected, the expression excited or dull, the tongue is coated, the lips and mouth dryer than normal; thirst, constipation, severe headache, pain in the back and limbs are complained of. Constipation is present in the great majority of cases, and often persists throughout the illness. By the time the patient is brought to hospital (fifth or sixth day) there is as a rule no doubt about the diagnosis. By the fifth day the mucous membranes are often implicated in the rash. The tongue has a well-marked white coat and is tending to become dry;

later the tongue is fissured, the mouth becomes offensive, sordes collect on the teeth, although these latter conditions can in many cases be prevented by proper nursing. Diarrhoea is not common in the early stage; about 20 per cent. of the cases develop it in the later stages. Asthenia and muscular debility are always present; patients can scarcely move in bed, they are often unable to protrude the tongue, and lose all expression. Emaciation in some cases towards the end of the disease is marked. In a small proportion of cases there is a definite crisis; in most cases, however, there is lysis, but a mixture of these beginning with lysis and ending in a crisis or the reverse may take place. The most common symptom of the real onset is headache, usually frontal, but sometimes mainly occipital. Conjunctival injection is present in four-fifths of the cases; it increases with the development of the eruption until lysis begins. Vomiting is present in about 25 per cent. of the cases. Sometimes it is severe and persisting for several days.

In many cases there is a distinctly reddish blush along the edge of the soft palate and pillars of the fauces, less frequently also slight congestion of the throat.

*The Temperature.* Whilst I believe it is possible to construct and describe what might be considered a normal temperature chart for an average case of moderately severe typhus, it is seldom that such a chart is met with in the wards in the natural course of the disease. A continuous or slightly remittent temperature of  $103^{\circ}$  or  $103.5^{\circ}$  F., during the first half of the second week, and then a slight daily decline until the eleventh or twelfth day, when there is a more decided remission with abatement of all the symptoms, is ordinarily what may be expected. Then there is another rise, say on the twelfth day, and a remission, and a second similar oscillation though less marked, with a decline to normal, and even a third, the whole lysis occupying forty-eight to sixty or seventy-two hours. Irregular temperatures are also met with. Again a definite crisis with a fall of temperature to normal in twenty-four or thirty-six hours may occur, although this is exceptional. After the temperature has dropped and the symptoms have disappeared, the drop may be to sub-normal for some days.

Of two hundred and forty-six cases that recovered, in nineteen the temperature was normal on the twelfth day, thirty-seven on the



thirteenth day, sixty-six on the fourteenth day, forty on the fifteenth day, and twenty-two on the sixteenth day.

*The Pulse.* Normally in ordinary cases, the pulse-curve follows that of the temperature. The pulse, however, is liable to show much variation; sometimes marked oscillations occur in the twenty-four hours, being at one time ninety and at another one hundred and twenty to one hundred and thirty in a minute. Dicrotism is not uncommon, especially towards the end of the second week. With the tendency to cyanosis so commonly seen in the late stage of the second week, the pulse is often absent at the wrist. The state of the patient's lungs appear to me greatly to affect the pulse, especially in wide-spread broncho-pneumonia. The pulse is markedly improved on the first signs of defervescence; in a few a very slow pulse is present in convalescence. The respiration curve varies less than that of the pulse.

*The Respirations.* In uncomplicated cases with moderate temperatures, the respirations are shallow and from thirty to thirty-five per minute, they vary little and without real dyspnoea at any stage. In similar cases with broncho-pneumonia, dyspnoea becomes a serious symptom.

*The Eruption.* This first appears on the evening of the fourth day in the form of discrete and well-defined pink or roseolar spots which may be round, oval or irregular, varying from 2 to 5 mm. in diameter, vanishing on pressure; they are seldom palpable at this stage, but they are widespread though scanty, and are seen on the abdomen, back, chest, shoulders, arms, legs and feet; they are rare on the face and head. In this early stage the rash described is not very obvious, it may require careful scrutiny to find it. The macules then become larger and of a bright red colour, next assuming a purplish-red hue running into dark purple. At this stage the tendency is for the eruption not to disappear on pressure, but this is not invariable—in many cases ending fatally with a deep coloured eruption before death, no sign of it remains *post-mortem*. When the eruption is fully developed on the eighth or ninth day, well-defined dark coloured patchial areas which do not disappear on pressure are seen, besides less-defined patches of much lighter colour which do not disappear on pressure. In all severe cases with typical eruption, these erythematous and patchial patches are met with

during the second week of the disease. In blonde boys and girls, during the early stage of the disease, we sometimes see on the chest, neck, arms, and occasionally on the abdomen, an irregular or blotchy erythema which vanishes before the real eruption is developed. In the second week the eruption has a multiform character—roseolar patches, red spots, maculae, small patchiae and large plaques typically patchial are seen; this multiformity is well seen on the shoulders and back, lower part of the abdomen and hips, outer surface of the arms and forearms, on which places what has been admirably named 'subcuticular mottling' is also visible. The eruption may, however, vary from consisting of only faint roseolar slightly raised spots to large ecchymotic looking patches. By the end of the second week little of the eruption is left. In some cases the general lousiness antecedent to the onset of the disease leads to considerable skin irritation with scratching and local secondary infections, which may initially be rather puzzling. Chronic pediculosis and pityriasis versicolor (both common in refugees) are the chief conditions of the skin likely to lead to confusion in a diagnosis based on the eruption alone. In about 1 per cent. of typhus cases there is either no eruption or only a faint roseolar one; this is more frequently the case in children and adolescents; in these cases the Wiel-Felix reaction is present. It is useful to carry about a good hand-lens, and, to bring out the eruption, rub into the skin some petrolatum; the lesions are then seen to consist of a congeries of dark red blood vessels.

Insomnia is one of the commonest symptoms; the majority of cases suffer from it during the first week of the disease.

In about 25 per cent. of the cases some form of mental disturbance is present on admission, and on the seventh or eighth day delirium. In cases that are running a fatal course, the delirium often passes into coma more or less complete. Cough is one of the most constant symptoms. In the earlier stage it is short and dry. Later on the expectoration may become profuse and mucopurulent. In a number of cases patches of lobular pneumonia occur. This is a common terminal condition in fatal cases. Diarrhoea is common in the later stages of the disease, and is then sometimes associated with rectal incontinence. Parotitis is one of the more serious complications; I saw altogether twenty of these cases, and as many as three in a

ward of thirty patients. Otitis media occurs in a small percentage of cases; it may become chronic. Deafness is a marked feature in many cases of typhus, during the late stage of the disease; this is quite distinct from the dullness of intellect that exists during that stage.

The spleen can be felt in about two-fifths of the cases; sometimes it is of considerable size. I do not lay stress on this, as many patients have a history of old malarial infection.

In all hospitals dozens of recurrent fever were sent in as typhus. In relapsing fever the *sudden* onset with rapid rise of temperature, severe headache, pains in the back and extremities, absence of dulness and apathy and (ordinarily) of rash, the presence of a moist tongue and of *S. obermeieri* in the blood, and later in the disease, more or less anaemia, should be sufficiently distinctive. In the Salonica Hospital the records show that eight cases of typhus and relapsing fever ran their course concurrently in the same persons.

The use of neo-salvarsan for the recurrent fever did not affect the normal progress of the typhus. The combined infection seems to suggest that the same louse may be able to carry the virus of typhus and *S. obermeieri* and inoculate them at the same time. Of course, two or more lice, each with a single infection, may have attacked these cases. It seems to be established that, contrary to the rule with intermediate hosts, the virus of typhus eventually kills the louse. In many, typhus and influenza ran together, the latter disease being also epidemic at the time. Hundreds of cases of both smallpox and typhus have been admitted into infectious diseases hospitals; in no single instance have both diseases been met with in the same patient at the same time; in one, typhus followed smallpox from infection acquired in the hospital.

*Wiel-Felix Reaction.* In typhus this reaction is very distinctive. It owes its origin to the discovery of the fact that what are called the 'X' strains of *B. proteus* are agglutinated by the serum of typhus cases. The special strains that do this are X2 and X19; these were primarily obtained from typhus urine. The macroscopic method is here usually adopted. The minimum dilution accepted as positive is 1 to 100. Practically the serum of every typhus fever case after the eighth day is positive, whilst that of other fevers is negative.

*Complications.* Bronchitis of greater or lesser severity is present in a large proportion of cases. Broncho-pneumonia is another common complication affecting the bases of both lungs. Pleurisy is much less frequent. Other complications are—severe diarrhoea, myocarditis, cardiac dilatation, parotitis, otitis media, conjunctivitis, keratitis, gangrene of the toes, bedsores, etc.

*Prophylaxis.* Medical men, nurses, and all sick attendants looking after typhus cases should be thoroughly protected from lice by suitable white cotton or linen clothing from head to foot before commencing their work, and have a bath and complete change of clothing after finishing their day's work. Even with these precautions infections will occur, but without them infection is all but certain, sooner or later, in those not immunised artificially, or by a previous attack of the disease.

*Etiology.* It would appear that the micro-organism of typhus passes through a development stage in the louse; in that insect it is intracellular, develops, is set free and is introduced into man. In man it is said to become intracellular once more, and from the infected cell to be thrown into the blood with its toxins. The members of the Typhus Fever Commission of the League of the Red Cross Societies to Poland, however, found no evidence of the development of the micro-organism in the louse, but they arrived at certain important conclusions as the result of their work.

Summarised, their conclusions are:—

*Pathology.* The lesions of typhus appear to be situated in the blood vessels of the skin, central nervous system, skeletal muscles, and to a lesser extent in some of the viscera—heart, kidney and testes. Typhus is considered to be a disease of the smaller blood vessels, and localises almost exclusively in the vascular endothelium. The reaction to the parasite is shown primarily by degenerative changes giving rise to thrombi in the blood-vessels, and by a proliferative reaction on the part of the endothelium and neuroglia which give rise to the characteristic 'nodules' of the disease in the skin and central nervous system. When lice are fed on typhus cases, while they develop *Rickettsia prowazeki* with great regularity they develop no other form of micro-organism. All lice so far do not become infective, but why this is so is not determined. Infection with *R. prowazeki* eventually kills the louse, which is an exceptional



effect of a parasite upon its intermediate host. *R. prawazeki* escapes from the alimentary tract with the faeces, and therefore may be introduced by scratching or by the mouth-parts of the louse becoming soiled with the faeces. *Rickettsia* has not been found in the salivary glands or in the mouth-parts of the louse.

*Mortality.* The average mortality in the infectious diseases hospitals near Athens is roughly 10 per cent.; it is, however, higher in some towns, such as Corfu, Patras, Volo, etc. It varies also greatly at different ages. In refugees of 50 years and over, the death rate is high, reaching in some towns 50 per cent.

It is necessary that some definite routine plan should be adopted in admitting typhus fever cases into hospital and distributing them in wards. The first requirement is a receiving-room, to which all patients are primarily brought. Here the hair of the head is rapidly cut off with a machine clipper, the hair of the axillae and pubes being shaved off; the hair is to be burnt. The receiving-room should communicate with the room or other area containing the steam-disinfector on the one hand, and with the bathroom on the other; this latter should lead to the dressing-room. After removal of the hair the patient is put on a stretcher and conveyed to the room containing the disinfector. Here he discards everything that he brings with him, which is disinfected. He is taken to the bathroom and bathed. He is then put on a clean stretcher and removed to the dressing-room; here he receives a suit of clean hospital clothing and is taken to the ward he is to occupy. There must be no remission in this routine; it must be thoroughly carried out if the wards are to be kept free from infected lice. Nothing that the patient brings with him to hospital should enter the ward.

Thoroughly deloused typhus cases are perfectly innocuous to the uninfected, and if we are quite confident as regards the efficiency of our delousing arrangements there is no reason for putting them in different wards.

*Treatment.* Typhus patients should be kept in bed throughout the pyrexial stage of the disease, and for a fortnight after the fever has subsided. Constipation is best relieved by a simple enema every second day. In some cases the catheter has to be used to drain off the urine. In the early stage, when sleeplessness, irritability, general discomfort and delirium are present, small doses of morphine



give satisfactory results. The morphine may later on be replaced by veronal, sulphonal, paraldehyde or chloral hydrate, if one or other is called for in mild delirium or insomnia. For prolonged and marked active delirium, hyoscine, hypodermically, is a valuable drug. The most popular stimulant is hypodermic injections of camphor (5 grains in 1 c.c. of olive oil or ether put up in ampoules); strychnine is also largely employed.

*Prophylactic Inoculations.* As a substitute for a prophylactic vaccine the blood of typhus fever cases has been inoculated, and the virus thus introduced in a living state. In using the living virus, Kusama injected monkeys with a fairly definite minimum dose of typhus blood, known as the minimal morbid dose, to bring about an attack. If a smaller dose is given no attack occurs, but a state of immunity is produced and the animal can tolerate many times the minimal morbid dose without ill-effects. This active form has so far not been used; the killed virus is the one that is used here. Whether it has any prophylactic value is uncertain.

*Prevention.* The amount of actual physical labour connected with the preventive work associated with typhus may be understood by describing what took place in two large blocks housing nearly two thousand refugees, in March last. From the 12th to the 17th, the whole camp was deloused and all bedding and clothing put through the steam disinfectors. The entire floors were washed with disinfectants and the walls whitewashed. This meant that every room had to be emptied of its entire contents while this was going on; then all the belongings of the refugees returned to the cleaned rooms, which were shut off from those awaiting their turn. It likewise meant giving a complete hot bath to everyone in the camp under supervision. From the 19th to the 24th there was a renewal of the bathing. A slight recrudescence of typhus after this necessitated a repetition of the processes carried out previously. This was done from the 26th to the 31st March. All men's and boys' heads were shaved, and all girls up to the age of fourteen had their hair bobbed. All cases of actual typhus, of course, had their heads shaved. Our nursing sisters also used on the heads of the refugees in camp the mixture of equal parts of kerosene and olive oil we employ to free nits from hair, and they distributed N.C.1 powder (naphthaline ninety-six parts, creosote two parts, iodoform two parts)

which was in little bags to be worn for a week, when a fresh bag is issued. The idea is that the heat of the body helps to vaporize the ingredients, and in this way is created a louse-destroying atmosphere next the skin and the clothes.

By order of the Government, refugees are allowed to leave the camps to work or to search for work, so long as their identity cards show that they are free from infectivity, which means free from lice. Many of the people allowed outside run the risk of acquiring the disease in other refugee camps which they visit, or elsewhere. This at present is unavoidable. Another weak link in the chain of preventive measures was the fact that for months after their arrival in Greece, a large percentage of the refugees did not possess a change of underclothes. It is obvious that where the people have only one suit of clothing there must be grave difficulties in rendering them lice free. An adequate supply of steam disinfectors for delousing clothes and bedding is almost indispensable; in their absence the task is most laborious. In small communities, Serbian barrels and other such improvisations may be useful, but in dealing with large masses of people they are futile.

I believe it to be well worth while in every camp to endeavour to educate the people in regard to the nature of typhus and the principles that underlie its eradication and prevention. We placarded leaflets in this connection, and also had them read out periodically for the benefit of the illiterate.

The specific measures now indicated appear to be to disperse the refugees from overcrowded towns to rural areas as much as possible. Another urgent requirement in many towns is an increased supply of water, which should be available to every house, or at least be within easy reach. In some camps the water-supply is decidedly defective, which interferes with the bathing arrangements, steam-disinfection of clothing and bedding and ordinary laundry-work—serious obstacles when endeavouring to eradicate typhus fever from an infected camp. The Greek Administration is endeavouring in all possible ways to remove this difficulty.

Every effort to quarantine contacts has failed, and all things considered it is not reasonable to expect it. All we want is that the person who is a contact should be watched definitely for twenty-one days, that is the time we laid down as the incubating

period. We endeavoured, as far as was possible, to render these certificated people free from lice; we felt that if thus free they could not communicate typhus, even if they acquired it themselves in the meantime. The quarantining of contacts in a small country which contains over a million refugees, many thousands of whom are contacts and are either in search of work or have daily to go to work from their camps and return to them, is an impossibility. It may be easy to do this in a limited area of infection with a stationary and disciplined population; it is an impossibility among refugees. The hope is that nature will step in during the summer and bring the epidemic to an end by stopping the multiplication of lice, by unfavourable meteorological conditions, and that by the beginning of the next typhus season the economic and other conditions will have altered for the better. At the present moment it is certain that the economic state of the refugees is decidedly antagonistic to effecting a cessation of the disease by the adoption of ordinary preventive measures.

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# THE VALUE OF THE SACHS-GEORGI REACTION IN THE SEROLOGICAL DIAGNOSIS OF SYPHILIS

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In order to determine to what extent the Sachs-Georgi could be relied upon to replace the Wassermann reaction in this country (Palestine), I commenced to perform a parallel series of tests in the latter part of 1921. It was by no means difficult to secure suitable material, inasmuch as it was possible to collect the sera of a large number of untreated cases which gave true clinical manifestations of syphilis in one or other of its various stages and forms. It was not so easy, however, to gather together sera from cases undergoing treatment, nor to follow up the effect of such treatment on the reactions.

Doubtless the disappearance of the then existing lesions led those affected into the belief that they were cured, and this deduction of 'out of sight, out of mind' is strengthened by the fact that only comparatively few names appear on the register more than once.

The conclusions arrived at, both from the performance of the ordinary tests and from certain experiments carried out bearing on the subject, are of interest, if, in one or two particulars, somewhat puzzling.

I shall in this paper detail:—

- (a) the methods employed—including the technique of the Sachs-Georgi reaction as carried out during the whole series; (which technique I have found by experience easiest and most suitable);
- (b) the actual results of the two reactions;
- (c) the relative percentage of agreement;
- (d) certain fallacies of the Sachs-Georgi reaction—certain experiments made with a view to explain the cause of several, and certain theoretical observations; and finally,
- (e) the conclusions and inferences drawn.



#### (A) METHODS EMPLOYED

For the performance of the Wassermann reaction, the Boas modification of the original has always been employed; the results of this technique have been so uniformly dependable that no other method is permitted in these laboratories. The antigen and haemolytic serum are both prepared by Burroughs, Wellcome & Co., and are forwarded every three months.

The technique of the Sachs-Georgi reaction is simplicity itself, and will be briefly outlined here.

(a) *Antigen.* This is supplied quarterly by Burroughs, Wellcome & Co., and although, in 1921, I experimented with various antigens, I found that prepared by the above firm so reliable that it was adopted in preference to the others.

A dilution of 1 in 20 is required.

1 c.c. antigen is pipetted into the ordinary 1 inch by 6 inches test-tube. To this is added 1 c.c. normal saline freshly prepared—the saline being allowed to run slowly down the side of the test-tube held in the sloping position.

The tube is agitated gently during the process of admixture. 18 c.c. normal saline are now to be added. The test-tube is held vertical and a pipette containing the 18 c.c. held with its point midway over the upper end of the tube. By gentle pressure on the indiarubber teat, the saline is allowed to fall drop by drop the height of the test-tube into the mixture; during the whole time this is being effected, the tube is gently shaken from side to side. The tube now containing 20 c.c. of 1 in 20 dilution is inverted slowly against the palm of the hand several times. The resultant antigen, ready for use, is a shimmering, somewhat opaque fluid with just a milky tint, giving the appearance of watered silk.

(b) *Patient's serum.* The serum is inactivated as in the Wassermann reaction, for half an hour at 56° C. When sera have to be sent to the laboratory from a distance, the medical officers are issued with instructions that blood must be taken with all aseptic precautions, and that the serum should be allowed to separate out in a sterile test-tube (preferably kept over night in the sloping position) and transferred to sterile bottles which are then carefully stoppered and sealed. In this way not only is haemolysis prevented



but also is obviated one of the chief causes of failure (to be detailed below) of the Sachs-Georgi reaction and discrepancy between this reaction and the Wassermann.

(c) *Method of setting up the reaction.* Four Wassermann tubes are used for each case, and dilutions of patient's serum made, 1 in 5, 1 in 10, 1 in 20, and 1 in 40, with normal saline freshly prepared.

Into tube 1 is placed 1.6 c.c. and into tubes 2, 3, and 4, 1 c.c. of normal saline.

To tube 1 is added 0.4 c.c. patient's serum and the contents of the tube are thoroughly mixed with a pipette. One-half the contents of tube 1 is transferred to tube 2 and thorough mixture made again.

To tube 3, one-half the contents of tube 2 is added, and again, after mixture, one-half the contents of tube 3 is transferred to tube 4. After suitable mixing, one-half of the tube 4 contents is now discarded.

Tube 1 now contains 1 c.c. of 1 in 5 dilution of patient's serum.

Tube 2 „ „ 1 c.c. of 1 in 10 „ „ „

Tube 3 „ „ 1 c.c. of 1 in 20 „ „ „

Tube 4 „ „ 1 c.c. of 1 in 40 „ „ „

To tubes 1, 2, 3, and 4 is added 0.5 c.c. of the already prepared 1 in 20 dilution of antigen, and the contents of all tubes are thoroughly mixed by inverting the tubes between the thumb and different fingers several times.

The tubes are now placed in an ordinary Wassermann bath and kept at a temperature of 37° C.

Mixing by inverting the tubes is done as a routine measure three times during the first twenty minutes, unless signs of a positive reaction have already made themselves manifest.

At the end of one hour, six hours, eighteen hours and twenty-four hours, the results are read and recorded. Saline negative and positive controls are set up in similar fashion according to the method described above. 1 c.c. of normal saline replaces the 1 c.c. patient's serum in the saline control, while 0.4 c.c. of known negative and positive sera will be added respectively to the 1.6 c.c. normal saline in the first tubes of the series instead of 0.4 c.c. of patient's serum.

For the positive control is taken one-half of a serum previously

proved to be positive both by the Wassermann and Sachs-Georgi tests.

#### READING OF RESULTS.

In well marked cases there is no difficulty, even for the most inexperienced, in reading both positive and negative reactions. The negative tubes show at the end of eighteen to twenty-four hours the same uniform shimmering fluid which on agitation presents the appearance of watered silk. The impression of the term 'watered silk' must be very carefully appreciated by the beginner, as upon that impression depends future success or failure in reading results.

The use of a hand-lens in doubtful cases is advocated and has proved of service to my assistants, but personally I have found the readings of 'granular positives' relatively simple, as my own visual acuity is - 5 D.

Positive results, if very marked, give the appearance of snow-flakes suspended throughout a clear fluid, and from the gradual settling of these white masses, a snowy layer ultimately forms at the bottom of the test-tube.

The supernatant fluid is then absolutely clear. The flocculations in this class are termed massive, and the precipitate heavy; we register it as XXX.

If definitely positive but less marked, the tubes give the appearance met with in ordinary bacterial agglutinations when a high titre serum is used.

The flocculi tend to deposit later and leave a clear supernatant fluid; this we regard as XX.

In the third group considerable difficulty may be experienced in differentiating the finely granular flocculi of a positive from the homogeneous suspension of the antigen. The appearance presented is somewhat like particles of dust shewn up by a ray of bright sunlight suddenly penetrating a dark room. There is little or no tendency for these particles to deposit, and the fluid does not become clear as in positives described above, this we record as X.

If doubt still exists, recourse may be made to having the tubes centrifuged to see whether a deposit can be obtained.

It has not been considered necessary to adopt any artificial lighting device to help in the reading of the results.

In passing, it may be remarked that on numerous occasions tubes

have shown what appeared to be positive reactions within the first few hours, but at the end of eighteen to twenty-four hours this appearance had completely disappeared; and also, it has been noted that gentle agitation may produce such a disappearance in what may be called pseudo-positives.

I shall have occasion later to refer to the occurrence of positive readings in the Sachs-Georgi reaction—actual positives which do not disappear as those mentioned in the last paragraph, but which owe their existence, although not to syphilis, to certain definite causes.

#### (B) ACTUAL RESULTS

##### 1. *Complete POSITIVE agreement was obtained between the Wassermann and Sachs-Georgi reactions:—*

(a) In three hundred and ten *untreated* cases submitted from venereal clinics and hospitals with a definite history of syphilis.

Analysis of these three hundred and ten cases:—

(1) *Primary stage*. Forty cases.

(2) *Secondary stage*. Two hundred and fifty cases.

These cases without exception showed typical pictures of secondary syphilis—rash, sore throat, mucous patches, etc. They had neglected the primary stage completely, and had come to seek advice only when the rash, fever, and constitutional symptoms manifested themselves.

(3) *Tertiary stage*. Eight cases.

TABLE I.

Cases	History	Wassermann reaction	Sachs-Georgi reaction
4	Gummata ... ..	XXX	XX
1	General paralysis ... ..	XXX	XX
2	Cerebro-spinal fluids in cases showing nervous symptoms	XX	XX
1	Hemiplegia ... ..	XX	XX

(4) *Congenital syphilitics*. Five cases.

These were discovered during the routine examination of inmates of an orphanage.

(5) In seven cases where the patients had several abortions.

TABLE II.

Case	History	Wassermann reaction	Sachs-Georgi reaction
1	3 abortions; 2 still births ... ..	XXX	XXX
2	5 abortions ... ..	XXX	XX
3	6 abortions ... ..	XXX	XX
4	5 abortions ... ..	XXX	XX
5	2 abortions ... ..	XXX	XXX
6	'Several abortions' ... ..	XXX	XX
7	'Several abortions' ... ..	XXX	XX

In case No. 1, husband had syphilis five years ago, and was treated with full course of Neo-Salvarsan injections. Serum tested by both reactions on same day as patient gave completely negative readings.

(b) In certain *treated* cases.

Eight cases showed markedly positive reactions. Of these cases one had received two injections of Neo-Salvarsan, and four had had 'complete' treatment (see below).

(c) In ninety-seven cases where no history accompanied the specimens.

## 2. *Negative agreements.*

Negative readings in all tubes of both reactions were obtained in five hundred and fifty cases of sera submitted without definite history, and as part of routine examinations. In two cases previously reacting XXX to both tests, sera now are completely negative to both.

## 3. *Partial agreements.*

These may be best illustrated as follows:—

TABLE III.

Cases	Wassermann reaction	Sachs-Georgi reaction	Remarks
4	XXX	X	1 with 4 injections of Neo-Salvarsan.



4. *Non-agreement.*

(a) In forty-five cases submitted, mostly without history, the Wassermann reaction was definitely positive, the Sachs-Georgi negative. In thirteen cases, however, details were supplied—symptoms of primary stage 8, of secondary stage 5.

(b) In twenty-one cases the Sachs-Georgi reaction was positive, the Wassermann completely negative. This figure does not include positive Sachs-Georgi reactions obtained during experiments performed. These latter will be detailed below.

(c) In twenty-three treated cases, the Wassermann was positive, the Sachs-Georgi negative.

(These cases were either partially or completely treated according to the following routine method practised here.

The course of Neo-Salvarsan injections is:—

1st injection	...	0.45 grammes.				
2nd injection	...	0.60 grammes one week later.				
3rd injection	...	0.75	"	"	"	"
4th injection	...	0.90	"	"	"	"
5th injection	...	0.90	"	"	"	" )

TABLE IV.

No. of cases	Wassermann reaction	Sachs-Georgi reaction	Remarks
14	XX	○	'partially' treated
2	XXX	○	'with 3 injections'
7	X	○	'complete treatment'

#### (C) THE RELATIVE PERCENTAGE OF AGREEMENT BETWEEN THE REACTIONS

The calculations are based upon one thousand and thirty-seven examinations of sera.

##### 1. *Positive agreement.*

In all, four hundred and eighty-seven showed a well-marked positive Wassermann, while with the corresponding sera the Sachs-



Georgi reaction showed positive readings in four hundred and nineteen.

The positive agreement therefore is 86 per cent.

2. *Negative agreement.*

Whereas the Wassermann reaction was negative in five hundred and fifty cases, the Sachs-Georgi was negative in six hundred and eighteen.

The negative agreement is therefore 89 per cent.

**(D) CERTAIN FALLACIES OF THE SACHS-GEORGI REACTION WITH CERTAIN EXPERIMENTS MADE WITH A VIEW TO EXPLAIN THE CAUSE OF SEVERAL, AND WITH CERTAIN THEORETICAL OBSERVATIONS**

(1) The presence in the serum to be tested of contaminating organisms renders the findings of the Sachs-Georgi reaction of no value whatever.

The first disparities between the Wassermann and Sachs-Georgi reactions here occurred in cases where the sera were sent from a distance, and when two or three days elapsed between collection and examination.

In those cases, fortunately, little doubt could exist as to contamination, as the sera were turbid and malodorous on arrival. Actual proof was obtained by plating on culture medium. A new series of reactions was performed in the case of those sera proved contaminated: they were re-submitted, and after transmission, arrived in a sterile condition. On these occasions they showed a completely negative reading where previously they had shown a strongly positive reaction. I refer in this connection only to sera which reacted positively to the Sachs-Georgi when the Wassermann reaction remained negative.

Further proof was adduced by the following simple experiment:—

A normal blood was drawn off in the laboratory, and the serum proved negative by both reactions.

The serum was then artificially infected with (a) *B. typhosus* and (b) *B. subtilis*, and then after suitable incubation the sera were set up in the ordinary dilutions, with the following results:—

TABLE V.

Case		Wassermann reaction	Sachs-Georgi reaction	Remarks
1	Normal blood serum ...	○	○	—
2	The same serum infected with <i>B. subtilis</i> ...	○	XXX	{ In both cases flocculation massive, precipitation heavy.
3	The same serum infected with <i>B. typhosus</i> ...	○	XXX	

If, then, the reaction could be completely altered by the presence of contaminating organisms in the serum, I considered it advisable to test the sera of patients suffering from certain diseases, to determine whether positive results in the Sachs-Georgi might be obtained here also.

The results are of interest, and although unfortunately at present the number of such examinations is small, yet I hope later to submit the results of examinations of many sera collected from patients suffering from the diseases most common in Palestine, so as to determine to what extent the Sachs-Georgi reaction is influenced by such.

First I was able to obtain six sera from lepers in Jerusalem, and the particulars and reactions are as follows:—

TABLE VI.

Case		Treatment	Wassermann reaction	Sachs-Georgi reaction
1	Nodular leprosy ...	Injections with <i>Ol. Chaulmoograe</i> for 1½ years ...	○	○
2	Nodular leprosy ...	Injections with <i>Ol. Chaulmoograe</i> for 3 months ...	○	X
3	Nerve leprosy ...	Untreated ...	○	XX
4	Nerve leprosy ...	Untreated ...	○	○
5	Nodular leprosy (?)	Untreated ...	XXX	XXX
6	Nodular leprosy ...	Treated with <i>Ol. Chaulmoograe</i> ...	○	XXX

From these examinations, no conclusions can be made.

In addition, however, the following examinations were made all at the same time with the fullest possible controls. All precautions were taken to ensure (a) freedom of the sera from contamination, except where otherwise indicated; (b) that the reactions were made in the dilutions considered essential; (c) that the reaction of the normal saline used was PH7; (d) that the readings were made after one hour, six, eighteen, and twenty-four hours, and that pseudo-flocculation was eliminated.

As these examinations have been performed for experimental purposes, the results have not been included in the calculations made as regards positive and negative agreements.

TABLE VII.

No. of case	History	Wassermann reaction	Sachs-Georgi reaction	Remarks
1	Street case taken at random ...	○	○	
2	Negative control ... ..	○	○	
3	Positive control ... ..	XXX	XXX	
4	Hospital routine examination ...	○	○	
5	Routine examination from clinic ...	○	○	
6	Case of untreated syphilis (stage 2)	XXX	XXX	
7	Sore throat, rash, typical 2nd stage	XXX	○	Disparity 1
8	Aneurysm of Aorta ... ..	XXX	○	Disparity 2
9	Case of syphilis previously reacting strongly to both reactions, treated with 4 injections of Neo-Salvarsan	XXX	XXX	Treated according to table above.
10	Case of syphilis previously proved positive to both reactions—treated with 4 Neo-Salvarsan injections	XXX	XXX	
11	Sore throat, mucuous patches ...	XXX	XXX	
12	Soldier—contracted syphilis 1921—fully treated ... ..	○	○	
13	Soldier—Syphilis 1921—full course of treatment ... ..	○	○	
14	Soldier—primary case chancre ...	XXX	XXX	
15	Soldier—ulceration of palate ...	XXX	XXX	

TABLE VII—Continued.

No. of case	History	Wassermann reaction	Sachs-Georgi reaction	Remarks
16	Male—previous syphilis untreated	XXX	XXX	
17	Female—married case 16 last year; has had a child full term, showing signs of congenital syphilis ...	XXX	XXX	
18	Untreated secondary stage syphilis	XXX	XXX	
19	Arthritis (right ankle) ... ..	XXX	XX	
20	Syphilis—after full treatment ...	o	o	
21	Periostitis of femur ... ..	X	o	
22	Ulceration of buttock ... ..	o	o	Pseudo-flocculation in first tube only.
23	Routine examination—no history	o	o	
24	Routine examination—no history	o	o	
25	Routine examination—no history	o	o	
26	Routine examination—no history	o	o	
27	Pharyngitis ... ..	o	o	
28	Chronic Jaundice ... ..	o	o	
29	Tumour of R. hypochondrium independent of liver; inguinal glands enlarged; pigmentation of legs and ankles ... ..	o	XX	Disparity 3
30	General malaise ... ..	o	o	Pseudo-flocculation in 1 tube only.
31	Pustular eruption ... ..	o	o	Pseudo-flocculation in 1 tube only.
32	Enlarged spleen (malarial) ... ..	o	o	
33	No history ... ..	o	o	
34	No history ... ..	o	o	
35	General malaise ... ..	o	o	
36	Serum from case of Typhus fever showing all clinical symptoms and reacting to Weil-Felix test ( <i>B. proteus</i> × 19) 1 in 400 ...	o	XXX	Disparity 4
37	Serum from case of Typhus fever Weil-Felix reaction 1 in 400 ...	o	XXX	Disparity 5
38	Serum from case of Typhus fever Weil-Felix reaction 1 in 200 ...	o	X	Disparity 6

TABLE VII—Continued.

No. of case	History	Wassermann reaction	Sachs-Georgi reaction	Remarks
39	Serum from case of Typhus fever Weil-Felix reaction 1 in 400 ...	XXX	o	Disparity 7
40	Serum from case of Relapsing fever	o	o	
41	Normal blood serum ...	o	o	
42	Serum of 41 infected with <i>B. typhosus</i> ...	o	XX	Disparity 8
43	Serum infected with <i>B. subtilis</i> ...	o	XX	Disparity 9
44	Serum of patient suffering from acute lobar pneumonia ...	o	XX	Disparity 10
45	Serum—another patient recovering from lobar pneumonia ...	o	XX	Disparity 11

In the case marked pseudo-flocculation in tube 1, it was found, as previously mentioned under 'reading of results,' that gentle shaking of the tubes in question produced an immediate disappearance of the seeming flocculi.

The importance of the two precautions—(a) always to shake the tubes gently before reading; (b) not to give definite reading until after the tubes have been set up for eighteen to twenty-four hours—cannot be too strongly emphasized.

I do not attempt to give any explanation of the disparities between the two reactions in the cases instanced above, but these few findings would seem to suggest that the presence of organisms, or products of organisms, in the patient's blood might well have some effect in reducing the value of the Sachs-Georgi unless the fullest history accompanies each serum submitted.

(2) Granted the complete sterility of the sera submitted, could any reason be advanced for the Sachs-Georgi reaction giving a strongly positive reading in the presence of a non-syphilitic serum?

From time to time in these laboratories the distilled water had been shown to be definitely acid, PH —, which acidity was due to various causes, principally carbon dioxide or absorption from an atmosphere containing acid fumes. Now it is a well-known fact that in agglutination tests, so-called 'pseudo-clumping' may occur on account of excessive acidity of the culture medium (see



Biggs & Park, American Journal of Medical Science, 1897; Block, B.M.J., 1897).

Agglutination of bacteria by acids in definite concentration can be carried out, and the phenomenon seems to depend upon the hydrogen ion concentration. In this connection the clumping of bacteria in acid agglutination is analogous to the clumping of colloidal suspensions of any kind, and the clumping or agglutination is merely a physical phenomenon, determined by the colloidal equilibrium of the bacteria in suspension. To digress a moment—this acid clumping was well exemplified by the experience of one of my assistants in Jaffa. He had been getting positive results in every Widal including controls performed in the laboratory there; and on enquiry being made it was discovered that the new laboratory attendant had rinsed the agglutination tubes after immersion in acid only perfunctorily. Reasoning that a similar or analogous phenomenon could occur in the performance of the Sachs-Georgi test, I had certain experiments carried out which seem to prove the likelihood of the supposition.

(a) Twelve sera were examined by the Wassermann and Sachs-Georgi tests. The diluent of antigen and serum was normal saline with a reaction of PH 7.

A third series was put up for the Sachs-Georgi test, but in this series the diluent of antigen and serum gave a reaction of PH 5.

The reading of the three series at the end of eighteen hours are as under :—

TABLE VIII.

No.	Wassermann reaction	Sachs-Georgi reaction (Normal saline used, PH. 7)	Sachs-Georgi reaction (Saline used, PH. 5)
1	XXX	XXX	XXX
2	XXX	XXX	XXX
3	o	o	XX
4	o	o	XX
5	o	o	o
6	o	o	X
7	XXX	XXX	XXX
8	o	o	X
9	o	o	X
10	X	X	X
11	o	o	o
12	o	o	XX
control			

(b) In the next experiment serum was completely omitted and the Sachs-Georgi antigen prepared with saline diluents showing a PH reaction of 5, 6·6, 7, and 8·5.

Four tubes were arranged with contents as under:—

Tube 1 contained 0·5 c.c. antigen (diluent PH 5) and 1 c.c. saline (PH 5).

Tube 2 contained 0·5 c.c. antigen (PH 6·6) and 1 c.c. saline (PH 6·6).

Tube 3 contained 0·5 c.c. antigen (PH 7) and 1 c.c. saline (PH 7).

Tube 4 contained 0·5 c.c. antigen (PH 8·5) and 1 c.c. saline (PH 8·5).

These were placed in the water bath at 37° C., and read at the end of eighteen hours.

Tube 1 showed marked flocculation and precipitation, while tubes 2, 3 and 4 remained without change.

The PH reaction of the saline here is usually 6·6, and it is found that this reaction in no wise interferes with the performance of the test.

(3) A phenomenon which forced itself early on my notice was that whereas a well marked positive reaction might be obtained in dilutions of patient's serum 1 in 20 and 1 in 40, no reaction whatever was visible in the primary dilutions 1 in 5 and 1 in 10 when the readings were made at the end of one hour, six, eighteen, and twenty-four hours. The occurrence was relatively frequent and demanded some explanation.

Here again one was compelled to seek a parallel in the ordinary agglutinations in bacteriology. And an analogy certainly exists. It must have been the experience of every bacteriologist in the reading of results of ordinary routine agglutinations to note that when an organism is set up against increasing dilutions of patient's serum, it occasionally happens that the serum in low dilution or greater concentration fails to agglutinate the organism, while with the serum in higher dilution or less concentration marked agglutination occurs.

This phenomenon in bacteriology has been accounted for theoretically by the 'pro-agglutinoid zone,' and the terms 'zones of no reaction' and 'zones of inhibition' have been applied to those dilutions wherein agglutination fails.

Briefly the pro-agglutinoid theory consists in the belief that for various reasons (*e.g.*, length of time elapsing between the collection and examination of the blood), the agglutinins called forth by any specific agglutininogen may deteriorate or become converted into substances capable of uniting with the agglutinogens without, however, resultant agglutination.

These substances have stronger affinity for the agglutininogen than the agglutinins themselves, and are termed 'pro-agglutinoids.' If these substances, then, are present in large numbers in strongly reacting sera, they may wholly mask the reaction by preventing the actual combination of agglutininogen and agglutinin. If, on the other hand, the serum is less concentrated, then in proportion is the number of these substances so lessened that they cannot have any appreciable effect in preventing agglutinin from uniting with agglutininogen, and therefore the reaction is not obscured.

It is not, perhaps, logical to strain the similarity between the two phenomena too far when it is to be remembered that the Sachs-Georgi reaction is not even a specific antigen-antibody one, the antigen being a homogeneous suspension of lipoidal substances, and the antibody (which bears probably no relation to true antibody), a lipotropic substance in syphilitic serum.

Similar phenomena, however, have been observed with non-specific agglutinating agents, and also in the action of coagulating agents on colloid emulsions.

Orthophosphoric acid, for example, will agglutinate a certain volume of a suspension of *B. coli* when present to the extent of between 118 cgrm. and 4 cgrm., and between 1.1 mgrm. and 0.001 mgrm., but not in intermediate amounts between 40 and 1.1 mgrm. (Hewlett).

Again, certain chemical substances have the power to agglutinate organisms, although their action is in no way specific, and the same substances will agglutinate different organisms (Beco). A mixture of equal parts of commercial formalin, alcohol, hydrogen peroxide, a 1 in 1,000 solution of chrysoidin, vesuvin, safranin, or perchloride of mercury, agglutinates the typhoid bacillus as well as other organisms. Whether, then, the phenomenon of zones of inhibition in the Sachs-Georgi reaction is determined by physical, chemical or other changes in the serum is not yet understood, but the analogy

between this and the phenomena occurring in ordinary routine agglutinations is at least very striking.

It is obvious, then, that if reliance were to be placed on the readings of the lower dilutions, or that if lower dilutions only (*e.g.*, 1 in 5 and 1 in 10) were to be put up, the results would be untrustworthy.

#### (E) CONCLUSIONS AND INFERENCES

1. The Sachs-Georgi cannot take the place of the Wassermann reaction, and should not be employed alone unless it is impossible to obtain the reagents necessary for the performance of the Wassermann.

2. The advantages claimed for the Sachs-Georgi reaction are:—

- (a) Negligible cost of reagents and necessities.
- (b) Simplicity of technique.
- (c) The rapidity with which strongly reacting positive sera can be read.

3. The Sachs-Georgi, from its percentage of agreement with the Wassermann Reaction (86-89), has a quite definite value, and if strict attention be paid by laboratory workers to the following points—fallacies (which constitute the main disadvantages of the reaction) may be largely obviated, and the Sachs-Georgi may be considered at least a useful aid in the diagnosis of syphilis:—

- (a) The patient's serum must be as fresh as possible and free from organismal contamination. If doubt exists as to sterility, cultural tests should be applied. (In this laboratory it has become routine practice to inoculate culture media tubes during the time the reactions are being performed, from all cases where the sera have been submitted from a distance and which might be likely to be contaminated. If growth occurs, the result is discarded.)
- (b) No opinion should be given on the results of this reaction unless a detailed history of the case accompanies the serum—this with a view to exclude the co-existence of other diseases. The presence of organisms, or their



products, in the patient's blood may completely negative the value of the reaction. In cases where a patient is suffering from an acute infectious fever, the reaction should not be employed.

- (c) The saline diluent of antigen and patient's serum must be freshly prepared, and its reaction very carefully estimated. Its reaction to PH should be 7, and a variation of not more than 6.6 to 7 allowed.
- (d) Not less than 3 (preferably 4) dilutions of patient's serum should be put up in each series, the last tube of the series showing a dilution of not less than 1 in 40—this to obviate the fallacy dependant on the 'zones of no-reaction.'
- (e) Final opinions should not be given until eighteen to twenty-four hours have elapsed from the time the reaction has been performed. Pseudo-flocculation, which may occur during the first few hours, tends to disappear before the end of twenty-four hours and, if still present, can be dispelled by gentle agitation of the tubes.

4. The treated cases which have been controlled throughout the full course tend to show that the Wassermann reaction remains positive longer than the Sachs-Georgi. A negative Sachs-Georgi reaction, then, in treated cases would not form a reliable index as to cure of the patient.

5. The Sachs-Georgi reaction remains negative in certain definitely established cases of syphilis, and this for no apparent reason. It is justifiable, therefore, perhaps to conclude that a negative Sachs-Georgi reaction is of little or no value.

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My thanks are due to Col. G. W. Heron, D.S.O., Director of Health, for his unfailing encouragement; to my colleague, Dr. R. Briercliffe, O.B.E., for controlling those readings which may be regarded as controversial; and to Dr. K. Krikorian and Mr. K. Daghljan, of the Central Laboratory, whose assistance and lively interest in the work have rendered the production of this article possible.



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# NOTE ON *AEDINUS AMAZONENSIS*, LUTZ

BY  
A. M. EVANS, M.Sc.

(Received for publication 1 August, 1923)

Amongst a consignment of mosquitoes collected on board s.s. 'Hildebrand' by Dr. A. Aiken Clark, during a voyage up the Amazon to Manáos, in 1922, was a male specimen of *Culex* with reduced palpi. It appears to be closely related to *Culex* (*Carrollia*) *paraplesia*, Dyar (1922), and comparison with the description of *Aëdinus amazonensis*, Lutz, suggests that it belongs to this little-known species.

*Culex*, sp. incert.

**MALE.** *Head.* Occiput with narrow curved creamy scales intermixed with pale golden upright forked ones above, with flat white scales at sides below and coarse golden setae projecting forward round eye margins. Palpi slender, about one-seventh the length of the proboscis, vestiture of pale brown scales. Proboscis bent at outer two-thirds, expanding distally (these two conditions probably due to accident), scales blackish brown, labellae yellowish. Antennae densely plumose, hairs of whorls blackish brown.

*Thorax.* *Prothoracic lobes* pale ochraceous, with a few flat whitish scales above and a row of blackish setae. *Mesonotum* with integument bright ochraceous, iridescent; in certain lights a narrow median dark stripe visible; vestiture of hair-like, bronze-coloured scales, with paler reflections, the scales in front of ante-scutellar space paler. *Scutellum* pale brown, with pale scales.

*Abdomen.* Dorsally blackish-brown scaled, with minute creamy triangular lateral spots on the last two segments. Venter largely denuded.

*Legs* with vestiture of blackish-brown scales.

*Wings* with first fork cell long and narrow, about four times as long as its petiole; second fork cell about twice the length of its

petiole. Scales on distal half of wing mostly of the type illustrated in the accompanying figure (fig. 1 A) widest beyond the middle, and with rounded apex. Scales on proximal half short, broad and truncated.

*Hypopygium* (fig. 2). Side-pieces (fig. 2 A) closely resembling those of *C. (Carrollia) paraplesia*, Dyar, but with a greater number of stout spines between the lobe (*l.*) and the clasper; apical part of lobe missing. *Mesosome* (fig. 2 B, C and D): lower bridge with small, highly chitinised, finely setose area (fig. 2 D, *b.*). Halves of mesosome consisting of thin plates of the form shown in the figures (fig. 2 B and C). In dorsal aspect the proximal portion (*p.*) is seen to give rise to a short inner portion (*i.*) and a much longer outer portion (*o.*), which constitutes the main part of the mesosome plate as seen in lateral view. *Ninth tergites* (fig. 2 E) about twice as high as broad; *tenth sternites* comb-shaped, with eight teeth.

It has recently been suggested by Dyar (1923) that the specimens described as *Culex originator*, Gordon and Evans (1922), from the Amazon Region, represent *Aëdinus amazonensis*, Lutz. I have, therefore, compared the types of *C. originator* with the description of Lutz's species and find that they differ in the colour of the thoracic integument, which is dark grey as described, and not ochraceous as in *A. amazonensis*; and in the character of the scales of the mesonotum and wings. The thoracic scales are narrow, but not hair-like as they are said to be in *A. amazonensis*, and there are no scales on the wings which could be described as 'Taeniorhynchus-like.' The main types of scales found on the apical part of the wing are illustrated in fig. 1; the scales on the proximal half of the wing are short and truncated. The specimen described above, however, agrees with *A. amazonensis* in these three particulars, the thoracic integument being of a conspicuously ochraceous colour. The only noteworthy difference appears to be the absence of a well defined median and fainter lateral dark mesonotal stripes in my specimen, which only shows a faint median stripe in certain lights. If, however, this character be subject to variation, it would appear highly probable that this *Culex* is *A. amazonensis*.

The structure of the side-pieces would seem to indicate a very close relationship with *Culex (Carrollia) paraplesia*, Dyar, but the latter differs in other hypopygial characters, the tenth sternites



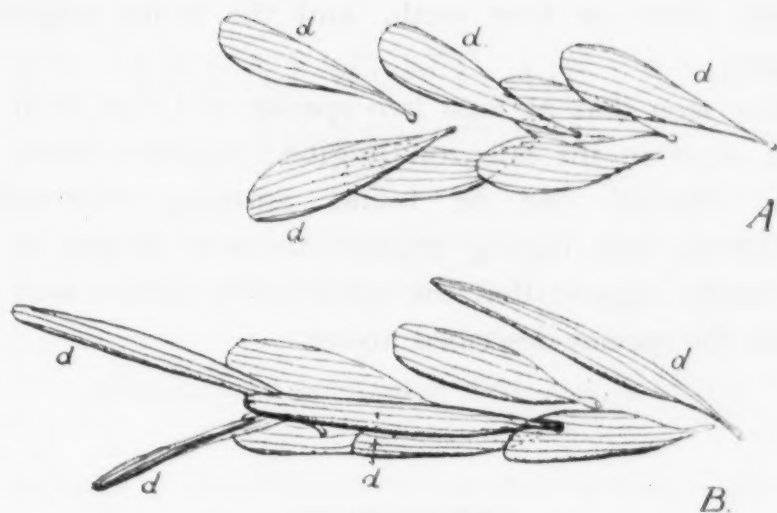


FIG. 1. Scales from upper branch of vein II. A.—*Culex* sp. incert; B.—*Culex originator*, Gordon and Evans. d.—scales of upper surface of wing.

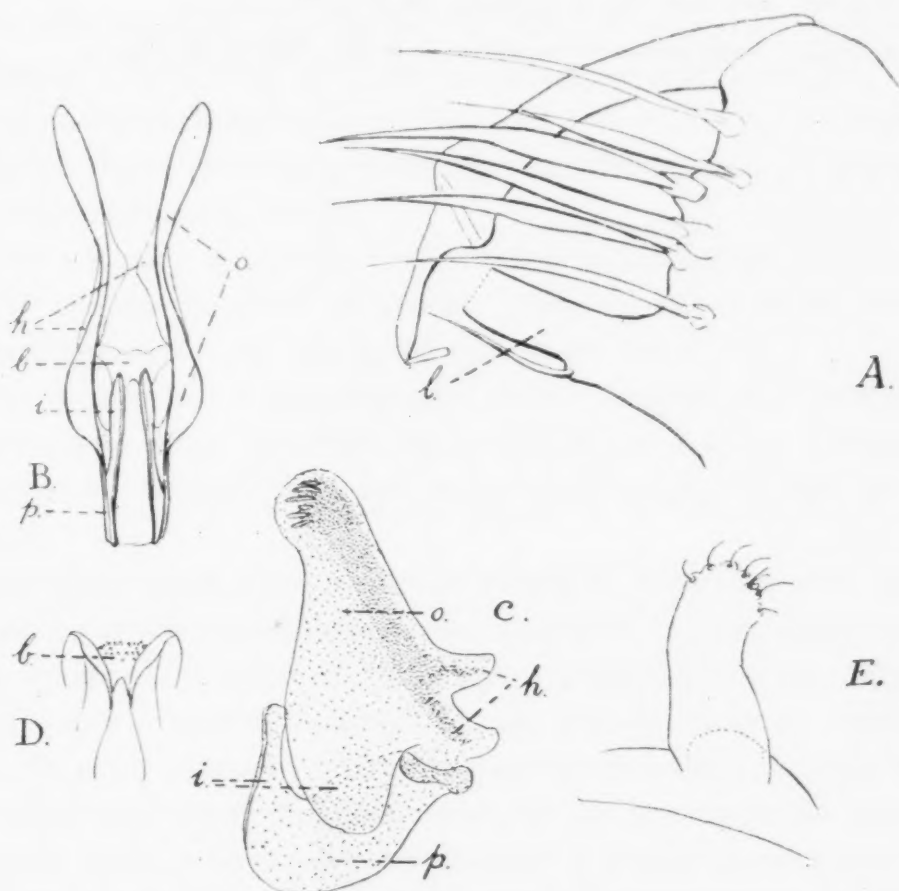


FIG. 2. *Culex* sp. incert. Hypopygium. A.—Side piece, in part with clasper. l.—lobe, broken distally. B.—Mesosome from above. b.—horns; i.—inner, o.—outer, b.—lower bridge.

having only three or four teeth, and the ninth tergites being undeveloped.

It is thus seen that at least two species of *Culex* with reduced male palpi occur in the Amazon Region; possibly others may be discovered. Should one be found agreeing externally with *A. amazonensis*, and having marked thoracic stripes as in that species, I would suggest that the name *Culex hildebrandi* be used to designate the species described above.

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## THE TREATMENT OF AMOEBIC DYSENTERY

BY

R. M. GORDON

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The majority of the cases recorded in the following paper were treated as in-patients at the Tropical Ward of the Royal Infirmary, Liverpool, their subsequent history after discharge being followed at the Tropical Clinic in the same city. At first it was hoped that much information might be gained by consulting the hospital and other records of the Ministry of Pensions, and through the courtesy of Dr. Finlay and others some four hundred case sheets of amoebic dysentery patients were examined. The results obtained were disappointingly meagre; a few of the cases are included in the tables that follow, but in the majority of instances the observation periods after treatment were too short to test the value of the drug given.

The following definitions were adhered to throughout:—

(1) The first diagnosis of amoebic dysentery was made by the finding of motile amoebae containing red cells in the faeces.

(2) Such a patient was considered to have relapsed after the completion of treatment when diarrhoea again occurred and active amoebae were observed in the stool, blood and mucus being usually, though not always, present.

(3) Cases which after treatment passed *E. histolytica* cysts unaccompanied by motile amoebae were not considered to have relapsed, but all such instances are recorded in the tables under the heading "Remarks."

(4) Once a patient relapsed he was considered as a "fresh case" and any other course of treatment was placed under a separate entry.

While undergoing treatment and for the first fortnight after treatment the stools were usually examined twice weekly (sometimes, as in the case of the emetine periodide series, much oftener) during the remainder of the observation period the examinations averaged about one a fortnight. In every instance, tests for the presence or absence of amoebae in the

stools were made by some member of the Liverpool School of Tropical Medicine.

No attempt has been made to compare the value of any two forms of treatment, as the observation period was not constant. Thus the relapses in treatments I and II were respectively 84 and 75 per cent., but if we fix an arbitrary limit of one month's observation and disregard all relapses occurring at a later date, then the relapses become respectively 53 and 17 per cent.

*Note on cases treated with emetine periodide.* Willmore (1923) records ninety-one cases of amoebic dysentery treated with emetine periodide of whom forty-eight (52 per cent.) relapsed. His observation period is similar to that used in the present paper, but his definition of a relapse includes persons passing *E. histolytica* cysts after treatment. Applying this definition to the sixteen cases recorded in Table I, treatment No. IX shows that ten of the sixteen cases (62 per cent) relapsed. Various vehicles for administering the drug were tried; formalised gelatin capsules were given in two cases of the acute type and all the motions passed in the subsequent twenty-four hours saved, by this method it was found that in both instances the capsules were passing through intact; even the plain gelatin capsules administered to patients with diarrhoea frequently passed through the gut without dissolving. Rice paper cachets were excellent, but owing to their brittle character sometimes allowed part of their contents to escape. At present we give the drug mixed with a little milk; taken this way it sometimes causes slight nausea but never vomiting. As Willmore's cases were all of the type that had 'proved refractory to all the known standard methods of anti-amoebic treatment' it appears of interest to record two cases of acute dysentery, one which (H.J.C.) had never received previous emetine treatment. Amoebae disappeared from this man's stool within forty-eight hours of the start of treatment and did not reappear during the six weeks he was kept under observation. The other case (C.M.P.) had received only one previous course of emetine (twelve grains emetine hydrochloride given fifteen months previously). During the first six days of treatment this patient continued to pass blood, mucus and amoebae, and at the end of this time his condition was so bad that it was thought advisable to supplement the periodide with four hypodermic injections of emetine hydrochloride. Under this combined treatment the amoebae vanished from the stools within twenty-

TABLE I.

Showing the effect of various forms of treatment on one hundred and thirty-eight cases of amoebic dysentery.

Nature of treatment		Observation period in months after completion of treatment							Total	Remarks
		1	2	3	4	5	6	more than 6		
TREATMENT No. I. Showing the effect of treatment with emetine hydrochloride gr. 1 given subcutaneously or intramuscularly two to six consecutive days.	Relapsing	7	4	...	...	...	...	...	11	One non-relapsing case was passing <i>E. histolytica</i> cysts four months after completion of treatment. Most of the cases in this series were of the acute type, i.e. passing a large number of blood and mucus motions in the 24 hours.
	Not relapsing	1	...	...	1	...	...	...	2	
TREATMENT No. II. Showing the effect of treatment with emetine hydrochloride gr. 1 given subcutaneously or intramuscularly ten to fourteen consecutive days.	Relapsing	5	4	1	3	...	2	6	21	One non-relapsing case was passing <i>E. histolytica</i> cysts 12 months after completion of treatment. The majority of the cases were of the acute type. Two non-relapsing cases were observed for more than a year.
	Not relapsing	1	2	...	2	...	...	2	7	
TREATMENT No. III. Showing the effect of treatment with emetine hydrochloride grs. 3 given by mouth on twelve or thirteen consecutive days.	Relapsing	10	4	3	...	2	...	6	25	Most of these cases were of the chronic type, i.e. passing a daily average of four to six loose stools containing active amoebae but little or no blood or mucus.
	Not relapsing	3	2	1	...	...	1	...	7	
TREATMENT No. IV. Showing the effect of treatment with emetine hydrochloride gr. 1 given subcutaneously together with fine bismuth iodide gr. 1 by the mouth on twelve consecutive days. (Three cases in ten days).	Relapsing	11	...	2	1	...	1	1	16	Two non-relapsing cases were passing <i>E. histolytica</i> cysts, respectively, 12 and 20 months after completion of treatment. The cases include about an equal proportion of acute and chronic types. Three non-relapsing cases were observed for 18 months.
	Not Relapsing	1	1	...	...	...	2	4	8	



TABLE I.—continued.

Showing the effect of various forms of treatment on one hundred and thirty-eight cases of amoebic dysentery.

Nature of Treatment		Observation period in months after completion of treatment.							Total	Remarks
		1	2	3	4	5	6	more than 6		
TREATMENT No. V. Showing the effect of treatment with pulv. ipecac. grs. 5, together with pulv. ipecac. Co. grs. 5 given by mouth on thirty consecutive days.	Relapsing	2	...	...	...	...	1	2	5	The majority of these were of the chronic type.
	Not relapsing	...	1	1	...	...	...	1	3	
TREATMENT No. VI. Showing the effect of treatment with pulv. ipecac. grs. 5, together with pulv. ipecac. Co. grs. 5 given by mouth on sixty consecutive days.	Relapsing	2	...	...	...	...	...	...	2	Most of these patients of the chronic type and repeatedly relapsed on various other forms of treatment.
	Not relapsing	1	...	...	1	...	2	3	7	
TREATMENT No. VII. Showing the effect of treatment with Ravauts paste, three drachms given by mouth on thirty consecutive days.	Relapsing	...	...	...	2	...	...	...	2	These were mild cases of a chronic type.
	Not relapsing	1	...	...	...	...	...	...	1	
TREATMENT No. VIII. Showing the effect of treatment with Ravauts paste, three drachms given by mouth on sixty consecutive days.	Relapsing	3	...	...	...	...	...	1	4	These were mild cases of a chronic type.
	Not relapsing	...	...	...	...	...	1	...	1	
TREATMENT No. IX. Showing the effect of treatment with emetine periodide grs. 6 given by mouth on thirteen to fifteen consecutive days. In one case, which is included amongst the non-relapsing, the emetine periodide was supplemented with four injections of emetine hydrochloride gr. 1.	Relapsing	8	...	...	...	...	...	...	8	Two non-relapsing cases passing <i>E. bistolytica</i> respectively, two and six weeks after the completion of treatment. The cases include an equal proportion of acute and chronic types.
	Not relapsing	1	2	2	2	1	...	...	8	

four hours and no relapse occurred during the five months the patient was kept under observation. Two other cases who had received numerous previous courses of emetine continued to pass motile amoebae throughout the time of treatment. Daily examinations of the remaining twelve cases showed that in two of them motile amoebae persisted for five days of treatment and in the other ten cases vanished after one to three days.

TABLE II.

Showing effects of various treatments not recorded in Table I.

Treatment.	Number of cases treated.	Result of treatment.
Emetine hydrochloride $\frac{1}{2}$ gr. given subcutaneously on two consecutive days.	1	Relapsed after six days.
Emetine hydrochloride $\frac{1}{2}$ gr. given subcutaneously on twenty-two consecutive days.	1	Relapsed seven months later.
Emetine hydrochloride $\frac{1}{2}$ gr. given subcutaneously on thirty consecutive days.	1	Relapsed within a month.
Emetine hydrochloride gr. 1 given subcutaneously together with emetine hydrochloride $\frac{1}{2}$ gr. given by mouth on ten consecutive days.	2	(1) No relapse after three months observation. (2) No relapse after three months observation.
Emetine hydrochloride gr. 1 given subcutaneously on six consecutive days followed by emetine bismuth iodide grs. 3 on twelve consecutive days.	2	(1) No relapse after twelve months observation. (2) Relapsed six months later.
Emetine hydrochloride gr. 1 given subcutaneously on twelve consecutive days followed by emetine bismuth iodide grs. 3 given by mouth on six consecutive days.	1	No relapse after twelve months observation.
Emetine hydrochloride gr. 1 given subcutaneously together with emetine bismuth iodide grs. 3 given by mouth on six consecutive days.	2	(1) No relapse after six weeks observation. (2) Relapsed a week later.
Emetine bismuth iodide gr. 1 given by mouth on twenty-four consecutive days.	1	Relapsed within a month.
"Yatren" 200 ccs. of a 5 per cent. solution given per rectum on ten consecutive days, then six days rest followed by a like dose for one day only.	1	Relapsed within a fortnight.

*Note on case treated with Yatren.* Mühlens and Menk (1921) recommend ten grammes of Yatren given by the rectum for eight to fourteen days, then no treatment for seven days; repeat the Yatren for three to seven days, allow another resting period of seven days and repeat treatment for three to five days. Owing to the fact that only a limited quantity of the drug was available, the course was shortened to that shown in Table II.

TABLE III.

Showing the distribution in various months of the numbers and percentages of one hundred and one relapses after treatment.

Treatment.	Relapses	Month in which relapse occurred after completion of treatment.						
		1	2	3	4	5	6	More than 6
Emetine hydrochloride gr. 1 given subcutaneously or intramuscularly on two to six consecutive days.	Number	7	4	...	...	...	...	...
	Percentage	63	36	...	...	...	...	...
Emetine hydrochloride gr. 1 given subcutaneously or intramuscularly on ten to fourteen consecutive days.	Number	5	4	1	3	...	2	6
	Percentage	23	19	4	14	...	9	28
Emetine bismuth iodide grs. 3 given by mouth on twelve to thirteen consecutive days.	Number	10	4	3	...	2	...	6
	Percentage	40	16	12	...	8	...	24
Emetine hydrochloride gr. 1 given subcutaneously together with emetine bismuth iodide gr. 1 given by mouth on twelve consecutive days (three cases only ten days).	Number	11	...	2	1	...	1	1
	Percentage	68	...	12	6	...	6	6
Pulv. ipecac. grs. 5 together with pulv. ipecac. Co. grs. 5 given by mouth on thirty consecutive days.	Number	2	...	...	...	...	1	2
	Percentage	40	...	...	...	...	20	40
Pulv. ipecac. grs. 5 together with pulv. ipecac. Co. grs. 5 given by mouth on sixty consecutive days.	Number	2	...	...	...	...	...	...
	Percentage	100	...	...	...	...	...	...
Ravauts paste three drachms given by mouth on thirty consecutive days.	Number	...	...	...	2	...	...	...
	Percentage	...	...	...	100	...	...	...
Ravauts paste three drachms given by mouth on sixty consecutive days.	Number	3	...	...	...	...	...	1
	Percentage	75	...	...	...	...	...	25
Emetine periodide grs. 6 given by mouth on thirteen to fifteen consecutive days (One case supplemented with four injections of emetine hydrochloride gr. 1).	Number	8	...	...	...	...	...	...
	Percentage	100	...	...	...	...	...	...
Various treatments recorded in Table II.	Number	5	...	...	...	...	1	1
	Percentage	71	...	...	...	...	14	14

The patient (A.G.) was a chronic case of about five years' duration who had completely resisted, or else relapsed shortly after, numerous forms of treatment. Before treatment commenced he was passing eight to ten motions a day containing blood, mucus and active amoebae. Twenty-four hours after the first rectal injection the amoebae disappeared and the number of stools were reduced to one or two a day. This condition lasted for twenty-eight days when blood, mucus and amoebae again appeared in the faeces. A further supply of the drug has been obtained and other patients are now under treatment.\*

### SUMMARY

One hundred and fifty cases of amoebic dysentery were given various forms of treatment and subsequently kept under observation for one to six months or longer; of these one hundred and fifty cases, one hundred and one (66 per cent.) relapsed, the numbers and percentages of the relapses occurring in various months being recorded in the tables. Amongst all the cases treated only six (4 per cent.) were observed to be passing *E. histolytica* cysts after treatment. Sixteen cases were given emetine periodide grs. 6 daily, eight of these (50 per cent.) relapsed within one month; the giving of this drug in gelatin capsules was found to be unsatisfactory as they frequently passed through the gut without dissolving; the periodide when mixed with a little milk and given by the mouth did not produce vomiting. Owing to the inequality of the observation periods no attempt was made to compare the value of any two forms of treatment.

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\* Since the publication of this note another case has relapsed, fourteen days after the completion of treatment. The patient in this instance had received the full course of treatment recommended by Mühlens and Menk.

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## RELAPSING FEVER IN THE GOLD COAST

BY

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The following account of an outbreak of relapsing fever in Accra, Gold Coast Colony, has been considered worthy of record since the disease has never before been recognised in the Colony.

Doubts have been expressed as to the possibility of the disease having been prevalent in the Colony in former years. For it is well known that circumstances tend to render the recognition of the disease unusually difficult since attacks may be so mild in character as to resemble slight attacks of malaria. Examples of this nature were met with during the outbreak to be described, and the danger of spread resulting from the movements of persons suffering from the ambulatory type of spirillar fever was readily appreciated. It is difficult, however, to understand how the disease could have existed undetected for, as can easily be understood, routine blood examinations in all cases of fever can be numbered in their hundreds every year, and had a proportion of these unclassified fever patients been suffering from relapsing fever, the spirochete could scarcely have escaped detection.

In connection with this it should be stated that Simpson discovered spirochetes in a single blood smear out of a large number of blood smears examined in 1908, the year of an outbreak of plague in the Gold Coast Colony.

### EPIDEMIOLOGY

Two possible sources of infection present themselves in connection with the epidemic to be described hereafter. One of these appears to have been the soldiers who returned to the colony after hostilities had come to an end in what is now called Kenya Colony. It is recorded that the West African forces, especially those in the

Dar-es-Salaam area, suffered severely from relapsing fever. It is possible that a certain number remained infective subsequent to their return to the colony and that infection spread to their families and to neighbouring tribes.

This theory is supported to some extent by the fact that the majority of the returned troops were members of the Northern Territory tribes and that many of these took their discharge from the Headquarters of the West African Frontier Force at Coomassie. The theory gains further support from the large preponderance of Northern Territory tribesmen, some of whom had seen war service, amongst the patients encountered during the outbreak at Accra. Moreover, a report has recently come to hand from the Medical Authorities in the Northern Territories of the Colony, to the effect that six cases of relapsing fever (confirmed by blood examination) have been isolated from recruits to the West African Frontier Force.

There are, however, several excellent objections to the acceptance of this theory, not the least of which is the fact that the West African troops while in East Africa suffered from infection by *Sp. duttoni* carried by the *Ornithodoros moubata*, whereas the strain met with in the Accra epidemic resembled *Sp. obermeieri*, in this instance the vector being the body louse.

The other possible source of infection may have been the neighbouring French territory, since a very extensive epidemic of relapsing fever was reported from Senegal and French Niger Territory in 1921.

Epidemics of relapsing fever are said to be slow in onset and to show a gradually increasing mortality. In the outbreak under review the onset was certainly rapid though its rapidity is slightly obscured in the chart (No. 1) owing to the artificial conditions resulting from emergency legislation. The mortality, moreover, fortunately showed no gradual increase in severity, as seen by the fact that the case mortality rate for the first fifty cases was 6 per cent., while that for the remainder was less than 1 per cent.

#### INCIDENCE

##### (a) Sex

As in India and other countries where the disease is endemic the large majority of cases occurred in adult males, the actual figures being one hundred and fifty-six male cases (including two European cases) and only two female cases.

This very large preponderance of male cases is not dependent upon the degree of lousiness of the two sexes, for observations showed that infestation was shared in equal measure by both men and women. The explanation, rather, lies in the fact that the infected males belonged almost exclusively to an immigrant tribe, who had come into the Accra district from their Northern Territory villages in search of work and money to purchase European goods. It is contrary to the customs and habits of these tribesmen to bring their women and children with them, and as they return to their native villages as soon as they have been able to collect together a small quantity of trade goods, they do not possess property and housing accommodation in Accra. It is not difficult to understand the conditions under which these people live in Accra, crowded together in insanitary hovels lacking in light and air.

(b) *Age*

Since the majority of cases of relapsing fever occurred amongst males of Northern Territory tribes, it follows that most of the cases would occur in adults, since the journey to Accra from the Northern Territories is not a thing to be lightly undertaken by persons other than healthy adults. The age of the patients varied from 10 years in a Hausa boy to 55 in a Zabramah man. The patients were almost all illiterate and consequently their ages could only be estimated approximately—the average being between 25-30.

(c) *Race*

The following table gives the racial incidence.

TABLE I.

Race or Tribe								Number of Cases	Percentage
European ...	...	...	...	...	...	...	...	2*	1.2
Kroo ...	...	...	...	...	...	...	...	2†	1.2
Kotokoli ...	...	...	...	...	...	...	...	3	1.8
Hausa ...	...	...	...	...	...	...	...	3	1.8
Other Tribes ...	...	...	...	...	...	...	...	9‡	5.6
Zabramah (Zaberrima) ...	...	...	...	...	...	...	...	139	87.9
Total ...	...	...	...	...	...	...	...	158	99.5

\* A European who became infected in the course of experiments, and who became infected a second time at a later date, is included in this figure.

† Two volunteers who were infected in the course of experiments.

‡ Includes a volunteer who was infected in the course of experiments as in the other three cases.

The reasons for the preponderance of Zabramah and other Northern Territory tribesmen amongst the cases are not hard to seek. Reports of extensive epidemics of doubtful character are not infrequently received from the Northern Territories and one such, accompanied by heavy mortality and attributed to cerebro-spinal meningitis took place in 1920. It is possible that relapsing fever may have been the cause of certain of these outbreaks and that shortage of medical staff allowed it to remain unrecognised. In any case an undoubted epidemic of relapsing fever occurred amongst natives in French Territory bordering upon the Northern Territories in 1921, and intercommunication across the frontier would account for infection.

Once infected, the habits of these tribes would ensure a rapid spread and perpetuation of the infection. Owing to a great scarcity of water in most parts of the Northern Territories, except in the wet season, the average tribesman from this area is brought up from birth in the belief that water is intended for drinking and cooking purposes only. In consequence his body, clothes, bedding and living quarters are quite innocent of soap and water.

This condition of affairs contributes to a state of lousiness, and infestation with lice is so general (100 per cent. of the Northern Territory patients admitted to the Contagious Diseases Hospital, Accra, were found to be lousy on admission) that little, if any, attempt at disinfestation is made on the part of sufferers from lice. Coastal tribes in the Contagious Diseases Hospital were amazed at the Health Authorities interfering with what was considered a 'custom of the country' when disinfestation of admissions was carried out. The fact that Northern Territory tribesmen and Hausas sleep in their work-a-day garments tends to add to the degree of natural lousiness.

In this connection it is a remarkable fact that coastal tribes, not excepting the Kroos, are singularly free from lice, due, no doubt, to the fact that they do not share the aversion from washing their bodies and clothes exhibited by Northern Territory tribesmen. In order to obviate the possibility of the importation of plague, smallpox and other infectious and contagious diseases into the Colony, all Kroo immigrants from the Kroo coast are medically examined on arrival at this port. Nine hundred and thirty-one were



so examined in 1922, and although they had been in most cases crowded together on board ship and had not had facilities for washing their bodies and clothes for as long as three weeks in some cases, not a single one was found to be lousy.

A further reason for the large proportion of Northern Territory tribesmen among the cases of spirillar fever lies in the insanitary conditions in which they lived in Accra. Not possessing any house property or relatives with satisfactory living accommodation, they crowd together in insanitary corrugated-iron structures lacking light and ventilation, intended by their unscrupulous owners not as living quarters but as stores for building materials and the like.

Lastly, owing to a temporary trade depression many of the Zabramahs were unemployed for some time prior to the commencement of the outbreak and, having neither friends nor relations in the district, they became half starved. The synonym 'famine fever' indicates the traditional association of semi-starvation and relapsing fever. With their powers of resistance so diminished they afforded fertile soil for the germ of any infectious disease.

The number of cases occurring amongst other tribes was too small to warrant conclusions being drawn as to the comparative severity of the disease, but it can be stated with fairness that the more severe type of case—and in fact all the fatal cases—occurred amongst Zabramahs.

#### (d) *Case Distribution*

A map is appended showing the districts in which patients appeared to have been infected.

Neglecting the first case which occurred in a European who had passed through a considerable number of bush villages in the Accra District during the course of his work, a number of the earlier patients appeared from information obtained from them to have been infected either in their native villages in the Northern Territories, or in one of the towns or villages at which they rested at night time, during their journey to Accra. This bears out the theory that the epidemic in Accra and District originated directly in Northern Territory tribes.

It will be seen from the map that the vast majority of cases occurred in the Tudu and Zongo Rd. areas of Accra, in both of which the inhabitants are almost entirely members of Northern



TABLE II.

Table to show location of places where patients are presumed to have been infected.

Place	Number of Patients infected
Accra ... ..	130*
Some village in Accra bush or on the road from the Northern Territories to Accra ... ..	19†
Nsawam ... ..	6
Mangoase ... ..	3
	158

\* Includes three African patients accidentally infected in the Colonial Hospital, and three Africans and one European infected experimentally at the Medical Research Institute (the European suffered from two attacks).

† Includes one European case infected in some unknown village in the Accra bush.

TABLE III.

Table to show areas in which cases of relapsing fever occurred, but not necessarily where infection took place.

	Number of Cases
Accra, Block No. 11 ... ..	1
Accra, Block No. 12 ... ..	19
Accra, Block No. 13a ... ..	43
Accra, Block No. 15a ... ..	8
Accra, Block No. 15b ... ..	50
Accra, Block No. 16 ... ..	13
Accra, Block No. 17 ... ..	3
Accra, Medical Research Institute ... ..	4*
Accra, Native Hospital ... ..	3
Accra Bush ... ..	6†
Residence unknown ... ..	8
Total ... ..	158

\* These cases were infected experimentally.

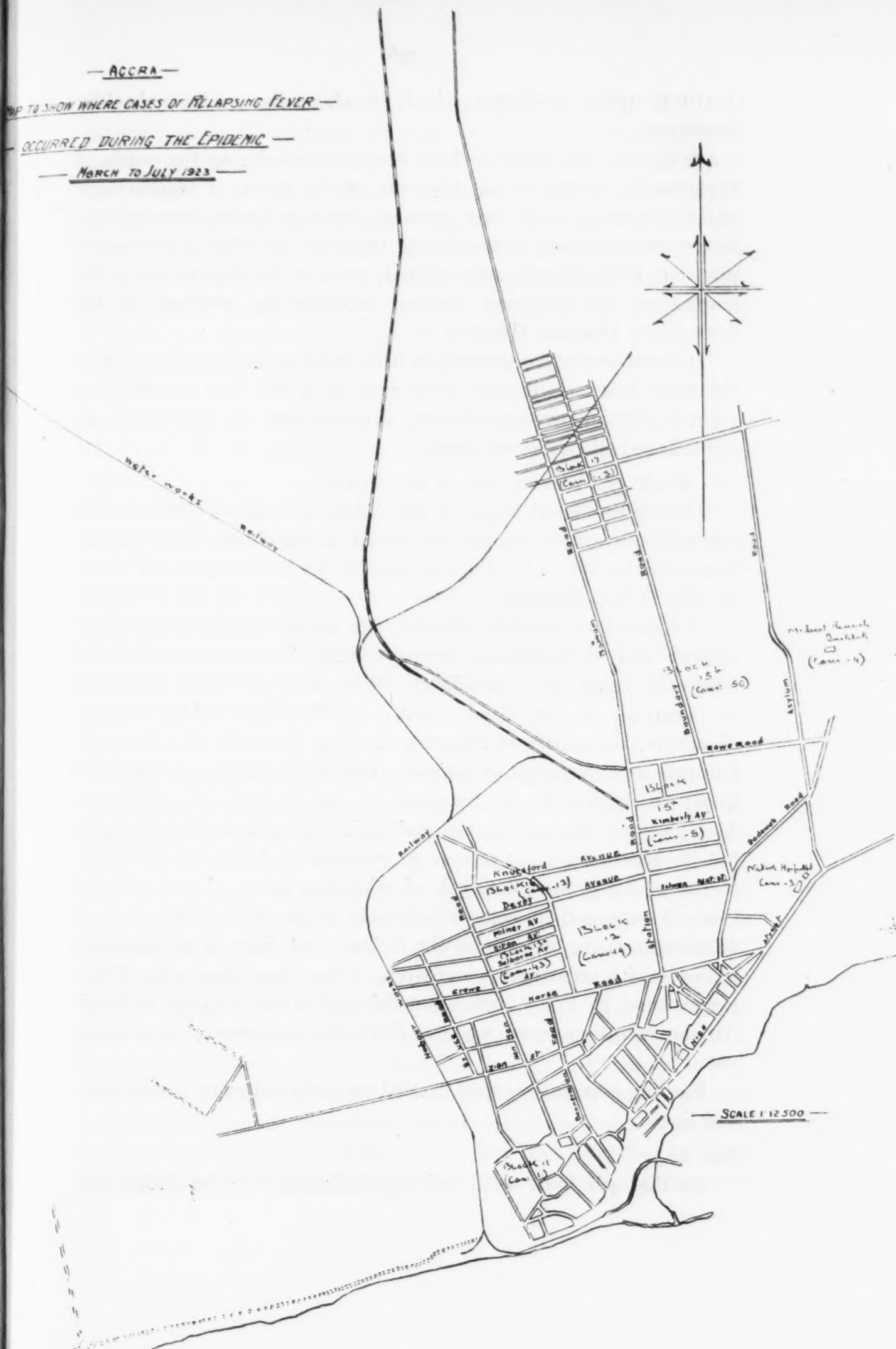
† This number includes a European, the first case to be discovered at the commencement of the epidemic.

— ACCRA —

MAP TO SHOW WHERE CASES OF RELAPSING FEVER —

OCCURRED DURING THE EPIDEMIC —

— MARCH TO JULY 1923 —



Territory tribes or Hausas—both of whom live under unhealthy conditions.

Four cases are shown to have been infected during the course of experiments aiming at the discovery of the means of transmission of the spirochaeta, while three cases are shown as having been infected in the general wards of the Native Hospital, in which a number of relapsing fever patients were treated, prior to the declaration of the disease as an infectious disease necessitating removal to the Contagious Diseases Hospital.

It is noteworthy in connection with the distribution of cases that the areas from which they came were of a very low standard as regards housing accommodation, overcrowding in insanitary, ill-lighted and ill-ventilated hovels.

(e) *Mode of Transmission of the Disease*

During the initial stages of the Accra outbreak of spirillar fever preventive measures against the spread of the disease were greatly hampered by the lack of knowledge of the exact means of transmission of the organism.

*Ornithodoros moubata* Murray, the carrier of tick fever in the Congo, and *Ornithodoros savignyi* Aud, the possible carrier of relapsing fever in Somaliland, have not yet been recorded as occurring in the Gold Coast. *Ornithodoros talaje* Guérin-Méneville, the carrier of relapsing fever in Panama, was taken by Graham on *Cricetomys gambianus* (Report on Plague in the Gold Coast in 1908: W. J. Simpson, p. 22), it was also taken on *M. decumanus* during the small epidemic of plague in Accra in 1917, but its occurrence is so rare as to preclude its being the prevalent carrier of the present outbreak of relapsing fever. *Argas persicus* Oken is supposed by some inhabitants of the Gold Coast to be a common parasite of fowls in the Colony, but there is no authentic record of its occurrence; moreover, it has been shown by Edm. Sergent and H. Foley (1922) that this tick is not a carrier of North African relapsing fever, whereas *Pediculus humanus* is an efficient one.

Feeding experiments were carried out with bed bugs, mosquitoes, and lice.

*Bed bugs*

On the 14th April seven bed bugs collected from the clothes and

bedding belonging to cases of relapsing fever which had been interned in the Contagious Diseases Hospital, were placed on the shaven back of a small monkey. At least two of these bugs fed on the monkey, but it was difficult to keep them in position so that the experiment was abandoned within four hours of its commencement. The monkey during the following fortnight showed no symptoms of illness and spironemata were never found in its blood.

A second consignment of bugs from the same source numbering a dozen was placed on the arm of a native volunteer on the 15th April, a modification of Nuttall's pill-box method of feeding lice being adopted. The bugs were retained on the arm for three days and were then removed and dissected, no spironemata were found in them and the volunteer never showed any symptoms of relapsing fever.

On the 30th April twenty bugs which had been collected from the bedding of relapsing fever cases were kept alive in the incubator for four days and were then ground up in saline. The coarser particles having been removed, the emulsion, which contained no spironemata, was rubbed into the scarified skin of a volunteer; the volunteer never showed signs of relapsing fever and spironemata were not detected in his blood at any time during the two weeks following the inoculation.

#### *Mosquitoes.*

*Aedes argenteus* Poiret (*S. fasciata*) being probably the most universally distributed mosquito in West Africa, and being the recognised carrier of *Leptospira icteroides*, was selected for experiment. Eleven female *Aedes argenteus*, reared from larvae, were placed in a gauze-covered jar and fed upon the arm of a patient suffering from relapsing fever in the Contagious Diseases Hospital before the patient had received any treatment. Five of the mosquitoes were seen to be engorged with blood when they were brought back to the laboratory on the 13th April. The mosquitoes were separated into two lots; six mosquitoes, of which three had certainly sucked blood, were placed in one jar and five, of which two were engorged with blood, were placed in another jar, honey and water were placed in these jars and the mosquitoes were kept alive with occasional renewal of the honey until the 24th April. Two volunteers began to feed these mosquitoes on their arms on that date



—(one jar being assigned to each volunteer)—and continued to feed them daily till the 2nd May when the mosquitoes began to die. No spironema was found in any of the surviving mosquitoes on dissection and neither of the volunteers showed symptoms of relapsing fever during the fortnight following the abandonment of the experiment, and at no time were spironemata found in the blood of either of them.

*Lice. (Pediculus humanus).*

On the 14th April four lice (*Pediculus humanus*), collected from the clothing of a case of relapsing fever, were placed by the aid of the pill-box method on the back of a monkey, but they refused to suck blood and were therefore transferred to the arm of a native volunteer: on the 17th April these lice were all found to have died. They were at once ground up in saline and the emulsion was inoculated into a black rat. Neither the volunteer nor the black rat showed any signs of illness and no spironemata were at any time found in the blood of either.

On the 30th April twelve lice were placed on the arm of a native volunteer; these lice had been found in the clothing of contacts with relapsing fever cases, nine of the lice were found to be dead on the following morning. The three surviving were then placed on a case of relapsing fever in the Contagious Diseases Hospital; after sucking blood from this case they were brought back to the laboratory, kept in the incubator for forty-eight hours, and were then placed on the arm of a second volunteer who managed to keep them alive for three days. Neither of the volunteers suffered in the least degree during the two weeks succeeding the feeding of the lice and neither showed spironemata at any time in his blood. The nine lice from this batch which were found dead on the arm of the first volunteer on the 1st May were ground up in saline and the resulting emulsion injected subcutaneously into a monkey without any effect following, the monkey remained well and spironemata were never seen in its blood.

On the 14th May a dozen lice obtained from the clothing of a case of relapsing fever in the Contagious Diseases Hospital were placed on the arm of a native volunteer and left *in situ* for seventy-two hours. At the end of that time ten were found dead, the two survivors together with the ten dead were ground up in saline and



the emulsion inoculated subcutaneously into a monkey. Neither the volunteer nor the monkey developed symptoms and spironemata were not found in the blood of either during two weeks following the experiment.

On the 16th May twenty-five lice were received from the Medical Officer of Health who had collected them from the clothing of cases of relapsing fever. These lice were divided into two lots and fed on the arms of two volunteers for three days with the same result as in previous experiments—neither volunteer developing symptoms of relapsing fever or at any time showing spironemata in his blood.

The droppings from these lice adherent to the sides and bottom of the test tube in which they were received were gently washed out with saline and the mixture rubbed into the scarified arm of a volunteer, but he developed no symptoms and his blood remained free from spironemata for two weeks following the inoculation.

On the 26th May two dozen lice collected from the clothing of several cases of relapsing fever in the Contagious Diseases Hospital were received and were placed on the arm of one of us (A.I.) by the aid of the pill-box method; these lice were fed at intervals during the day and night till the 4th June when, there being only six survivors, they were ground up in saline and the emulsion which contained spironemata was inoculated into the scarified arms of two native volunteers. On the 12th June one of the volunteers complained of headache and lumbar pain, his temperature was found to be  $100.2^{\circ}\text{F}$ . and spironemata were found in thin and thick films of his blood. On the 13th June the other volunteer reported sick, his temperature was  $100.6^{\circ}\text{F}$ . and his blood showed spironemata. No spironemata were present in the blood of the individual upon whose arm these lice were placed at any time; it was examined from the 26th May to the 4th June whilst the lice were being fed.

On the 4th June four lice were supplied by the Medical Officer of Health (P.S.S-C.). These lice had been allowed to remain on a patient suffering from relapsing fever for five days after he had been treated with Novarsenobillon. When received at the laboratory the lice were placed on the arm of a native volunteer using the modified pill-box method; they were left on this individual's arm for twenty-four hours only and one of them had disappeared when the pill-box was removed. The three survivors were ground up in

saline and a portion of the emulsion was rubbed into the scarified arm of another volunteer, a second portion was dropped into the conjunctival sac of a monkey, while the residue was inoculated subcutaneously into a second monkey. This emulsion contained spironemata. Neither of the monkeys became ill and during the two weeks following the experiment no spironema was found in the blood of either. The second volunteer into whose arm the emulsion had been rubbed suffered from headache, and had a temperature of  $99.2^{\circ}\text{F}$ . on the 14th June, but no spironemata were found in thick films of his blood examined daily for a week after the slight rise of temperature. The first volunteer upon whose arm the four lice were placed began to be ill on the 15th June, but did not report himself as sick until the 18th June, when his temperature was found to be  $102^{\circ}\text{F}$ . and spironemata were numerous in thick films of his blood.

On the 11th June twenty lice from the clothing of cases of relapsing fever in the Contagious Diseases Hospital were supplied by the Medical Officer of Health (P.S.S-C.); these were at once placed on the arm of a native volunteer who fed them for three days. On the 14th June, however, as he seemed reluctant to continue to feed the lice, the survivors, five in number, were transferred to the arm of one of us (A.I.) where they were fed for another four days. During the last period of feeding two of the lice escaped one night from under the bandage retaining the pill-box in position and wandered freely over the body—judging from the number and position of the bites discovered the following morning. These lice were not recovered and may have been crushed in an attempt to allay the irritation of their bites. On the 18th June the feeding of the three ultimate survivors was discontinued and they were ground up in saline and the resulting emulsion, which contained spironemata, was inoculated subcutaneously into a monkey. This monkey showed no symptoms of illness and spironemata were not at any time found in its blood, which was examined daily till the 2nd July. The native volunteer who fed this batch of lice for three days immediately following their last meal of infective blood remained free from sickness, and no spironemata were found in his blood which was examined daily for twelve days after he had discontinued feeding the lice. The second individual who continued the feeding of the

lice became ill on the 25th June, his symptoms were headache and pains in the limbs and his temperature was  $101.6^{\circ}\text{F.}$ , but no spironemata were found in a thick film of blood; on the morning of the 26th June when the temperature was  $102.4^{\circ}\text{F.}$  a few spirone-mata were seen in a thick film of blood, they became more numerous at a later stage of the attack.

It may be of interest to mention that the individual who became sick as the result of this last feeding experiment was the same who developed relapsing fever as a result of accidental inoculation on the 28th March. It is possible that this second attack may have been a relapse of the former, but it appears to us more probably a reinfection, the interval between the recovery from the first attack and the onset of the second, a period of eleven weeks, being too great; besides at no time during the interval were any spironemata found in the blood which was repeatedly examined, and no symptoms of illness were experienced.

These feeding experiments appear to agree with the conclusions of the French observers in Tunis and Algeria, Ch. Nicolle, L. Blaziot et E. Conseil (1912), and Edm. Sergent et H. Foley (1922), namely, that the disease is not conveyed by the bites of lice or by their droppings being rubbed into excoriations of the skin, but that it is conveyed by the inoculation of crushed lice into wounds of the skin; further, that lice must be kept alive for about one week after feeding on a case of relapsing fever before they are capable of conveying the infection.

The two last experiments may seem rather equivocal, suggesting that the infection is conveyed by the bites of lice alone. It is to be noted, however, that in both experiments one or two lice escaped from the pill-box in which they were enclosed and wandered over the body generally; it is therefore quite possible that these stray lice were unconsciously crushed and rubbed into abrasions of the skin made in the process of scratching.

The pill-box method of feeding may not be an ideal method for use in the tropics, as pointed out by Cragg (1922), but when properly applied it certainly prevents crushing of its contents on the skin by any attempts at scratching.

Up to the time of writing, experiments have not been carried out to show whether infection is transmitted by infected lice to their

eggs, though this has been shown to be the case in other parts of the world.

It is noteworthy that lice were found on a very large proportion of all the patients treated at the Contagious Diseases Hospital, more especially among Zabramahs and other Northern Territory tribes and Hausas, in whom lice were found on the hair of the head, beard, axillae, pubic region and on wearing apparel.

The louse appeared to resemble the head and body louse found in Europe—*Pediculus humanus L.* It was remarked that lice tended to migrate from an individual having a high temperature, suggesting that the optimum skin temperature was probably in the neighbourhood of 98.4°F. or less. The temperature of the air and the relative humidity did not appear to influence the numbers or habits of the louse.

A graph and table given in the appendix illustrates the lack of influence exercised by temperature and humidity on the numbers of cases discovered in Accra from week to week.

(f) *Time of Occurrence of Cases*

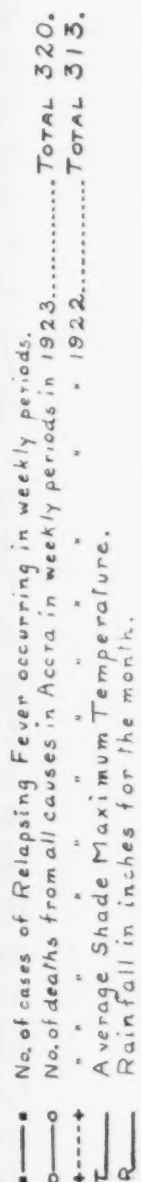
A chart is appended to show the progress of the epidemic from week to week. A somewhat erroneous impression is gained from an inspection of the chart, however, since the peak of the outbreak would appear to have occurred in the weekly period April 29th—May 5th. The large excess of cases occurring during this and the following weekly period resulted from legislation being passed on April 28th, which allowed the Health Authorities to round up nearly four hundred suspected cases and contacts with cases isolated from certain areas.

Briefly, a steady weekly increase in the number of cases reported occurred from the first case on March 18th, attaining a maximum during the weekly period April 29th—May 5th, and then steadily decreasing until only one case occurred during the weekly period June 24th—30th, this case being a second attack in a European infected experimentally in order to confirm the mode of transmission of infection.

(g) *Meteorological*

A daily maximum temperature of over 98.8°F. is stated to exert an unfavourable effect on lice. Conclusions based upon observations







carried out over a period of three months showed that the small variations in atmospheric temperature and relative humidity had little or no influence on the degree of infestation of the Northern Territory tribes normally found infested with lice.

The outbreak commenced towards the end of the dry season during March, when the average maximum shade temperature was 88°F. and the relative humidity 67.

The greatest number of cases occurred at the end of April and at the commencement of May, during which times the rainy season had been in progress for a short time, and the average maximum shade temperature and relative humidity for April and May respectively being 88°F. and 86.5°F. and 67.1 and 76.9. The epidemic virtually came to an end early in July during the continuation of a rather more than normally wet season. The slight fall in temperature and the decided increase in rainfall between March and July appeared to have little influence on the course of the epidemic or upon the severity of individual cases.

(h) *Morphological Characters of the Spironemata*

The spironema found in the blood of cases met with in the present epidemic differs in no way from the descriptions given of the spironemata causing relapsing fevers in other parts of the world. Two hundred spironemata taken as they came—twenty-five in eight blood films from separate cases—were drawn with the help of the camera lucida and measured by the compass method, Macfie and York (1917). The shortest spironema found measured  $10\mu$  and the longest  $44\mu$ , the average length being  $21.9\mu$ . The commonest lengths of the spironemata were  $18\mu$  to  $23\mu$ , and the average thickness of the spironemata was  $0.3\mu$ . The pleomorphism noted by the French observers, J. Kerrest, A. Gambier et A. Bouron (1922), in the Soudan epidemic of relapsing fever has also been noticed by us, but it appears to us to be merely a passing phase; in blood films obtained from the same case on consecutive days, we have found few, if any, irregular forms on the first day, while on the second day ring and figure-of-eight forms have been numerous and did not require to be searched for. Breinl (1908) states with regard to *Sp. duttoni*, that coiled and complicated skein-like forms are most numerous in the blood of the internal organs just before the crisis sets in. Balfour and Bousfield (1911) have described and

figured these irregular forms of spironemata in relapsing fever at Khartoum. With the exceptions of ring, figure-of-eight and partially coiled forms, the shape of the organism did not appear to undergo any change in patients from day to day and, although the majority of the films examined were air-dried before being treated with Ruge's fluid, the exposure of blood films to hot air, to the vapours of formalin, to osmic acid, or to chloroform, appeared to have no influence on the shape of the organism.

The spironemata found in emulsions of crushed lice appeared to be shorter and more delicate than those seen in blood films, they also stained less deeply with gentian violet.

The number of organisms found in thick blood smears varied from over forty per field observed in a case which resembled in many respects a typical case of lobar pneumonia to as few as two over the greater part of the slide.

It would have been anticipated that spironemata would have been more numerous in severe cases of the disease and in first attacks than in relapses, but this was not invariably the case, although as a general rule they were less easy to find in relapsing cases and in fact were rarely found in what appeared to be a relapse after injection of a substerilising dose of Novarsenobillon. In one case spironemata were found by Dr. Mary Magill (who kindly assisted to examine a group of nearly two hundred films prepared from contacts and suspected cases) in a blood film of a contact who was not suffering from pyrexia at the time nor for the forty-eight hours intervening between his blood being taken and his treatment with Novarsenobillon. This case showed no signs or symptoms of illness and was discharged fourteen days subsequent to his receiving an intravenous injection of 0.3 gm. of Novarsenobillon, not having shown any signs of sickness. This blood film was one amongst twenty-five other films of contacts, all of whom appeared and were healthy; thus the possibility of the slides having become mixed could be excluded.

It is a remarkable fact that a careful search through blood films taken from some patients who appeared to be suffering from typical attacks of spirillar fever, who were stricken at the same time as their comrades, and who reacted to intravenous medication in exactly the same way as their fellow patients, failed to show the presence of infecting organisms.

In this connection it is noteworthy that in the severely collapsed cases with subnormal temperatures spironemata were not discovered in thick films until reaction had set in and the temperature mounted to 100°F. or more. Owing to the system adopted of taking the temperatures of all contacts and suspects and of carrying out a routine blood examination of every person segregated, whether he suffered from pyrexia or not, conclusive evidence was obtained as to the absence of the organism in the blood in the apyrexial state with the sole exception of the case described above. Spironemata were not found in the specimens of sputa and urine obtained from relapsing fever patients.

#### ANIMAL EXPERIMENTS

The following animals were inoculated with blood obtained from cases of relapsing fever at the Colonial Hospital or at the Contagious Diseases Hospital, Labadi:—White rats, black rats, *Cricetomys gambianus*, guinea-pigs, monkeys and one rabbit. The quantity of blood inoculated varied usually from 0.5 ccm. to 2 ccm., citrated blood being employed in all but one of the experiments. The rabbit and guinea-pigs proved refractory, no spironemata being at any time found in their blood, which was examined daily for a fortnight after inoculation. Eight white rats were inoculated at different times with infected blood, but in only one of them were spironemata seen; this rat was given 2 ccm. of blood from a human case on the 21st March and on the following day, twenty-six hours after the inoculation, two spironemata were found in a thin film of its blood; on no other occasion in this rat were spironemata found, although the blood was examined daily for a fortnight. That the blood employed in the cases of two of these white rats inoculated on the 28th March was infective was proved by the inoculator unwittingly infecting himself and developing relapsing fever, the first symptoms of which appeared on the 4th April—seven days after infection occurred.

Three monkeys were inoculated. Monkey No. 1 received a few drops of serum only, as the blood, obtained from the first case diagnosed, was carelessly allowed to clot in the syringe; this monkey never showed any symptoms and spironemata were never detected in its blood, which was examined daily for a fortnight after inoculation.

Monkey No. 2—a small baboon—was given 2 ccm. of citrated blood containing spironemata on the 21st March. On the 23rd it was not so lively as usual; on the 24th it had a temperature of 102° F. and spironemata were numerous in its blood; they were less on the 25th, and had disappeared completely on the 26th. From this day onwards to the 6th April, when the daily examination of the blood was discontinued, no spironemata were found. This monkey, which has been under close observation for three months, has never shown symptoms of a relapse.

Monkey No. 3—a sooty mangabey—was inoculated with about 2 ccm. of blood containing spironemata on the 23rd April. It appeared rather subdued on the 25th, but otherwise showed no symptoms of being ill, and its temperature was only 100° F.; on the 26th spironemata were numerous in its blood, but had disappeared on the 27th, and after this date no spironemata were found in its blood. This monkey has also been under close observation for nearly three months and has shown no signs of relapse.

Three black rats (*M. rattus*) were inoculated. Two received about 1.5 ccm. of citrated blood on the 28th March; this was the same sample of blood which failed to infect two white rats but proved infective in the case of the inoculator. Neither of these black rats showed spironemata in its blood, which was examined daily for twelve days following the inoculation. A third black rat was given 4 ccm. of citrated blood from a human case on the 2nd July. Spironemata were fairly numerous in its blood on the 4th, but were absent on the 5th, and have never been found since that date.

Two *Cricetomys gambianus* Waterhouse were given large doses (4 ccm.) of citrated blood which contained spironemata on the same occasion as the black rat last mentioned. On the 4th July spironemata were found in the blood of one of them; on the 5th both showed spironemata in large numbers in thick blood films; on the 6th the blood of the first rat which showed spironemata on the 4th July was free from them, while that of the other showed them in large numbers; subinoculations were made from each of these rats into another rat of the same species on this date. Both subinoculated rats showed spironemata, but at different dates after inoculation; the rat receiving blood containing spironemata showed them in its blood on the third day, the rat receiving blood which was apparently



free from spironemata on the eighth day. The original two rats relapsed, one after its blood had been negative for four days, the other after its blood had been negative for seven days.

A white rat was subinoculated with blood from Monkey No. 3 when it contained numerous spironemata on the 26th April, with a view to finding if passage through a monkey exalted the virulence of the strain for white rats. The blood of this rat never showed spironemata on any occasion, though examined daily for a fortnight after inoculation.

The results obtained from these inoculations appear to correspond closely with the inoculation experiments conducted by Gambier (1923) at Bamako. Gambier found monkeys to be readily infected with spironemata, white mice to be infected with difficulty, and rabbits and guinea-pigs to be refractory.

*Cricetomys gambianus*—the pouched rat—showed itself to be much more susceptible to infection with spironemata than any of the other animals employed; it was the only animal which appeared to relapse. It should be possible to convey the strain to Europe by means of a series of these rats provided they can be got to survive the rigours of a northern climate.

#### CLINICAL MANIFESTATIONS

The first case recorded was I. A., an Italian contractor, aged 26. He was admitted to hospital on 15th March, 1923, with pyrexia, headache and severe prostration. He had been resident in the Gold Coast Colony two years, engaged in contracting work, and had enjoyed good health. He was taken ill on March 14th. His blood, on admission, was found free from parasites and pigment. It was examined again on March 17th, when it was found to contain numerous spironemata. On March 18th he was given 0.3 gm. Novarsenobillon intravenously. The following day, being the sixth day of the disease, the blood was found free from spironemata, and the temperature dropped to normal, where it remained until the patient's discharge from hospital on March 24th. There was no relapse, and six weeks later I. A. sailed for Italy, apparently in perfect health. The other symptoms in the case were icterus of the sclerae, severe pains, especially in the thighs, vomiting, enlargement

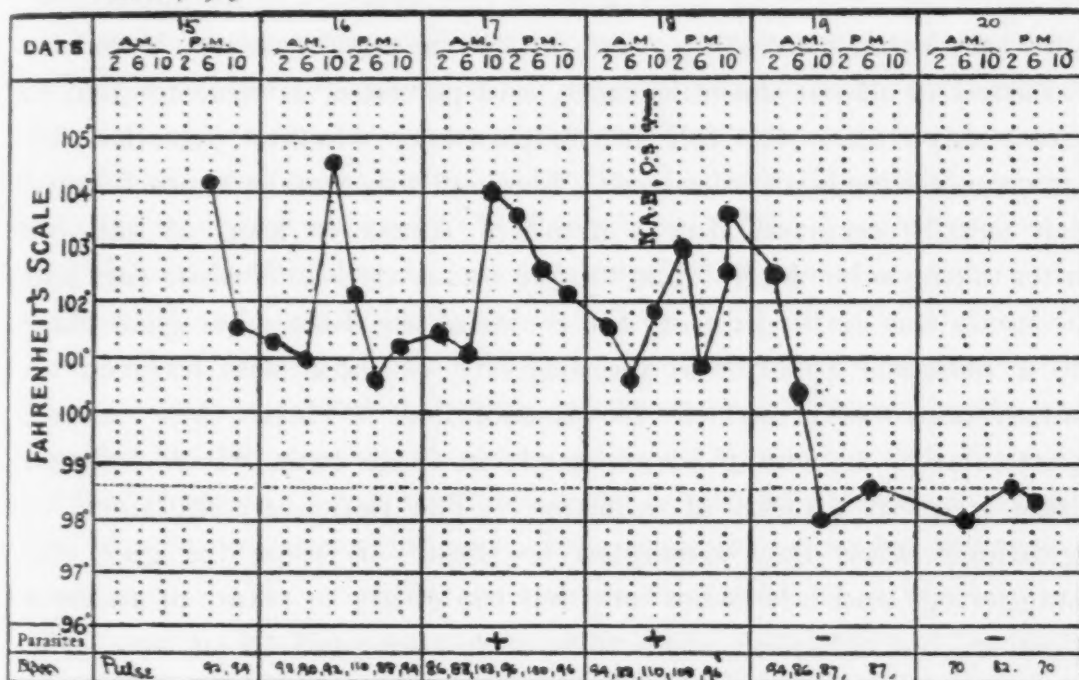


of the spleen, and a severe nephritis. The urine contained albumen and a heavy deposit of granular casts; all this cleared up by March 23rd.

The only other European case was accidentally infected on two occasions. His illness ran a similar course, excepting that jaundice and nephritis did not occur. In this case the rheumatic pains, especially in the thighs, were excruciating. The second attack was quite similar to the first, and occurred after an apyrexial period of three months. It was much milder in character. Both attacks were treated with the intravenous injections of 0.3 gm. Novarsenobillon, and there were no relapses.

I. A., Italian, Male, Age 26. Relapsing Fever. Admitted 15th March.

March, 1923



The clinical manifestation in natives presented a much more varied picture.

Owing to the necessity for the employment of one and sometimes two interpreters in the case of Northern Territory patients, it was far from being an easy task to obtain reliable information regarding symptoms.

The incubation period was not definitely established, but appeared to vary from seven days in the case of an accidental infection resulting from spirochetes in a drop of blood from a

patient, entering the system by way of a bruised nail bed, to twelve days in the case of another volunteer who allowed himself to be bitten by infected lice, and who is thought to have scratched a louse into his skin. In the case of two other volunteers, infection took place within eight or nine days of their receiving an emulsion of crushed lice rubbed into the scarified skin.

Prodromal symptoms were rare. The onset of the disease appeared to be sudden, and was often accompanied by a distinct rigor. Frontal headache became so marked in some cases as to warrant description by the patient as a pain like 'hammering on the temples.'

Some of the patients complained of severe pains in the cervical and lumbar regions, in thighs, shins and wrists. Prolonged attacks of shivering, rendering the taking of temperatures quite impossible, and very profuse sweating, were noticed in certain cases. Vomiting occurred in all but the mild cases, and persisted in some for two to three days after the fall in temperature, whether preceded by intravenous medication or not. The vomiting was at times bilious, but usually occurred after a drink of Akasa—a kind of pap—or after taking other food. The tongue was coated with white fur, and anorexia was marked during the course of the disease but gave place to a ravenous appetite in the majority of those who received an intravenous injection of Novarsenobillon. Thirst was severe, particularly, as would be expected, in those cases which suffered from frequent attacks of vomiting. The patients complained of giddiness when they attempted to stand; in some the gait was staggering, and others not only felt too giddy to stand in an erect posture, but collapsed when they even attempted to sit up. The asthenic condition persisted in some after convalescence had been established.

Both liver and spleen were enlarged in many cases, but as the patients for the most part came from malarial infected areas, it is possible that malaria was the cause of the splenomegaly, although diminution in size of the spleen was recorded during convalescence (quinine not being administered). Jaundice was present in remarkably few cases. In one series of one hundred and seventeen, only three patients suffered from jaundice.

The majority of the patients suffered from constipation, but a

certain number, particularly those in whom the temperature had fallen by crisis, suffered from diarrhoea.

The urine showed albuminuria during the pyrexial stages of the disease, but was not remarkable for any peculiarities. Oedema of face and hands, suggesting a nephritis, was seen in a small minority of cases.

Cough and a small degree of bronchitis were present in many of the cases, and in one pulmonary signs were so marked as to lead to a provisional diagnosis of pneumonia being made. Except in this case the pulse-respiration ratio was normal.

Mental symptoms were observed in a certain number of cases, and varied from a slight vacuity of mind and loss of memory to profound mental dulness and, in a small number, to active delirium and a comatose condition.

The pulse rates recorded were for the most part consistent with the height of the temperature in the pyrexial period, although in three cases out of a series of one hundred and seventeen in whom convalescence was prolonged owing to cardiac dilation, the rate remained unduly rapid for two weeks or more after the fall of temperature to normal. In the collapsed cases the pulse was of poor volume, thin and thready and often uncountable.

A somewhat unusual series of facts were noted in connection with the temperature recorded. It might have been assumed with all fairness that the severity of other signs and symptoms in a case of spirillar fever was proportionate to the height of the temperature. This was by no means the case. In some cases where the temperature rose to  $104^{\circ}\text{F}$ . or higher the symptoms were by no means severe, and recovery rapidly took place after suitable treatment. In other cases where the temperature did not rise much higher than  $100^{\circ}\text{F}$ . other signs and symptoms were grave. In the single case under the care of one of us (P.S.S.-C.) which ended fatally the temperature on admission was  $99^{\circ}\text{F}$ . A thick blood smear was taken and large numbers of spironemata were observed. The patient was given an intravenous injection of 0.6 gm. Novarsenobillon and then put to bed. His temperature rose to  $101^{\circ}\text{F}$ . by 6 p.m. the same evening. On the following morning his temperature had fallen to  $99^{\circ}\text{F}$ ., but his condition was grave and he could not be persuaded to take any fluid nourishment. A blood smear was negative to spironemata.

He vomited bilious-looking material twice during the day, and by 6 p.m. his temperature was  $99.4^{\circ}\text{F}$ . When seen on the following morning at 6-30 a.m.—probably the fourth day of his illness—he was found to be comatose. The thermometer did not register any temperature and his radial pulse was so small in volume and rapid in frequency as to be almost imperceptible and quite uncountable. A blood smear was negative to spironemata. Efforts were made to combat the condition by raising the end of his bed, by applying hot blankets, hot water bottles and by administering intravenous and subcutaneous salines and brandy. By 10-30 a.m. his temperature had risen to  $97.2^{\circ}\text{F}$ . and his pulse, though rapid (120), was of good volume and tension. Respirations which had been of the Cheyne-Stokes variety at 6-30 a.m. were now comparatively normal although the breathing was stertorous. By 2-30 p.m. the patient's temperature had risen to  $101^{\circ}\text{F}$ . His pulse was of good volume and tension and about 120. Breathing had become markedly stertorous and respirations numbered 36 to the minute. A blood smear was taken but no organisms were found. The patient died at 4 p.m. on the same day, having remained unconscious for over 48 hours. As far as could be gathered, the patient had been ill for two days prior to his admission to hospital, thus death took place within five days of the initial symptoms. The above case has been given at some length in order to show that the height of the temperature had little relation to the severity of an attack. It is to be noted that the blood smears taken on the second and third days of the illness prior to the patient receiving Novarsenobillon showed a very heavy infection of spironemata.

Another type of temperature was seen in the case of a female subject of good physique and aged 20. The temperature recorded on the first four days of her illness was  $100^{\circ}\text{F}$ . or less. A blood smear taken on the first day proved on examination to show a moderate number of spironemata. On the second day—the patient remaining without treatment—the number of organisms in a thick film was very small, and on the third day none were found. By the fifth day the temperature had fallen to  $97.4^{\circ}\text{F}$ ., and it remained low for ten days. On the tenth day following the initial fall to below normal, the temperature rose to  $100^{\circ}\text{F}$ . On the following morning at 6-30 a.m. the temperature was  $104.4^{\circ}\text{F}$ . Spironemata were not



found in the blood until the eleventh day following the original commencement of the apyrexial period after the first attack. Owing to the obvious suffering of the patient and to her serious condition, one of us (P.S.S.-C.) did not feel justified in withholding Novarsenobillon any longer and gave 0.6 gm. intravenously at 11 a.m. By 6-30 p.m. the temperature had fallen to 103° F. On the following morning the temperature still stood at 100° F. but fell to 99° F. the same evening and to 97° F. by the next morning. Spironemata were found in moderate numbers at the height of the relapse but not subsequent to the treatment with Novarsenobillon.

In a second patient—an adult male aged 44—untreated until the first relapse, the temperature on admission on the third day of his illness was 103.2° F. Spironemata were present in moderate numbers in a thick blood smear. The patient received no treatment other than a cold sponging, and his temperature fell the day after his admission to 97° F., remained normal or subnormal for two days and then rose to 101° F. During the apyrexial period, organisms were absent from blood smears, but were present on the day of the relapse. The patient appeared to be suffering considerably during the relapse, and it was not considered fair to him to withhold specific treatment any longer. The temperature fell to normal and spironemata disappeared from blood smears within twenty-four hours of the patient being injected with 0.6 gm. Novarsenobillon and no further relapse occurred.

Particulars of a fourth case are worthy of record since the patient appeared to be suffering from pneumonia on admission. The patient was admitted to hospital on the second day of the disease. His temperature, pulse and respiration at 2-45 p.m. on the day of admission were respectively 103.6° F., 100, and 40. Although a well nourished male of 25 he was too weak to move hand or foot and was delirious. Bronchitic râles were heard over both sides of his chest and signs of early pneumonic consolidation were heard over the left lower lobe. A blood smear showed the presence of spironemata in large numbers. The patient was very jaundiced. He was given 0.6 gm. of Novarsenobillon intravenously and his temperature, which rose to 104° F. by 6 p.m. the same evening, fell to 100° F. on the following day and then to 97° F. on the morning of the third day after his admission. Subsequently, the temperature rose again on



the evening of the third day following admission to 99·8° F. and to 100° F. on the morning of the fourth day, but fell to normal on the same day. From thence onwards the temperature went to a few points above normal for the next fourteen days and then steadied down to subnormal. The pneumonic signs cleared up without any signs of resolution, but the patient suffered from bronchitis for a fortnight following his admission to hospital. Spironemata were not found in the patient's blood subsequent to the treatment with Novarsenobillon.

When discussing the variations in temperature in cases of relapsing fever it would be unwise to take the four cases quoted above as typical examples. By far the majority of cases suffered from temperature varying from 100° F. to 105° F., though a small number showed spironemata in blood smears with a temperature of only 99° F. In most cases a fall of temperature to normal or subnormal took place within twelve hours of treatment with Novarsenobillon, although in some cases the temperature remained above normal though lower for two to three days and then fell. During the early days of the epidemic when only 0·3 gm. of Novarsenobillon was administered, a number of the patients relapsed after varying intervals. In a small minority of cases it was found necessary to give 0·6 gm. of the drug followed by 0·3 gm. after an interval of three days.

#### RELAPSE

The information regarding the occurrence of relapses is scanty for two reasons. Africans, and in this they resemble all races, do not take kindly to hospital treatment, and purely medical cases prefer to remain in their own homes rather than to enter hospital, however comfortless the former may be, and however much their chances of recovery may be so impaired. It follows naturally that if, owing to pressure having been brought to bear on them, they have been admitted to hospital for treatment, their one aim and object is to obtain their discharge therefrom as soon as possible. Consequently, when a case of relapsing fever was admitted to hospital, endeavours were made to sterilise the patient as regards the infecting organisms in his blood and to effect his cure with the least possible delay. By making his stay in hospital as short as was compatible

with his own well-being and with the safety of the general public, other cases occurring in the town were encouraged to seek medical attention as soon as they became infected, instead of remaining concealed from the health authorities and so helping to spread infection.

Secondly, it was not considered justifiable, except in a very small number of cases, to withhold treatment from a patient with a view to determining the approximate length of the apyrexial period between attacks, since by so doing, the well-being and possibly even the life of the patient was placed in jeopardy.

The relapses observed can be divided into two classes, one in which the patient had received no specific treatment, but only general symptomatic treatment as, for example, light diet, saline purge, cold sponging, and the second class in which specific treatment with Novarsenobillon had been administered.

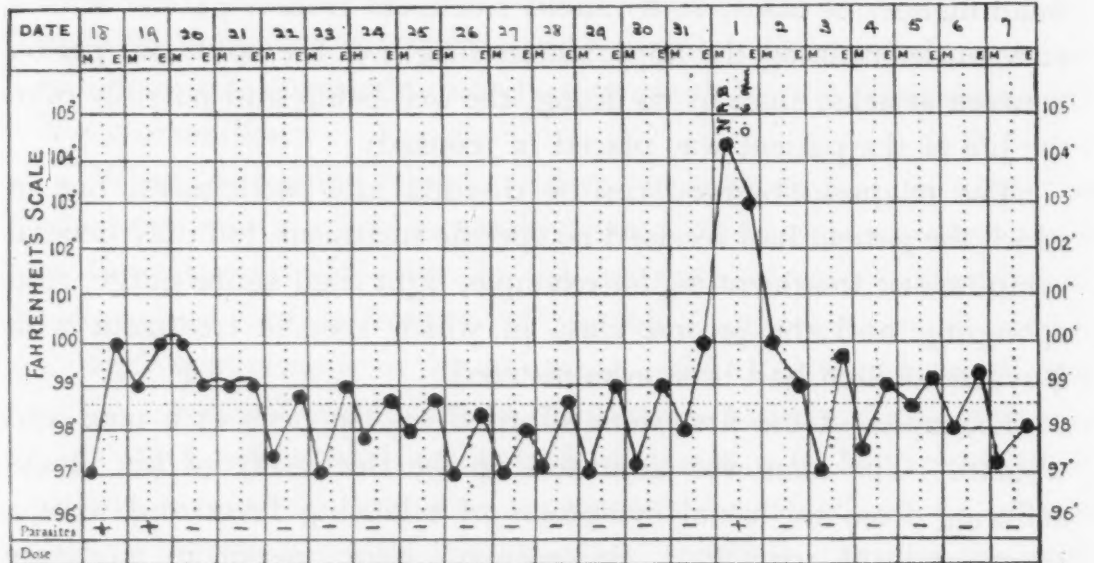
Examples of the first were afforded by the cases of a man and woman. The man was admitted on the third day of his illness suffering from the usual symptoms of relapsing fever and with a temperature of  $103.2^{\circ}\text{F}$ . Spironemata were present in moderate numbers in a thick blood film. The patient received symptomatic treatment only and his temperature fell to  $97^{\circ}\text{F}$ . on the day following admission and remained normal or subnormal for two days. On the third day following the crisis which terminated the first attack, the temperature rose to  $101^{\circ}\text{F}$ ., and spironemata which had been absent from blood films during the apyrexial period of two days were again found in blood films. The condition of the patient did not justify the withholding of treatment further. The woman suffered severely from headache, pains in the back, legs and wrists, but her temperature during the first four days of her illness did not exceed  $100^{\circ}\text{F}$ ., although a blood film taken on the first day showed a moderate infection with spironemata. The number of spironemata seen on the second day of the disease was smaller, while none were found on the third and fourth days. On the fifth day of the disease the temperature fell to  $94.4^{\circ}\text{F}$ . and remained low for ten days. On the tenth day following the initial fall to below normal the temperature rose to  $100^{\circ}\text{F}$ . and on the following morning at 6-30 a.m. to  $104.4^{\circ}\text{F}$ . Spironemata were found in moderate numbers, but the patient's condition was sufficiently serious to make further delay in the administration of specific intravenous medication quite unjustifiable.

Several examples of the second series of relapses, that is to say, relapses occurring in spite of treatment, occurred amongst the cases

ARMAH, Female, Age 20. Relapsing Fever. Admitted May 18th.

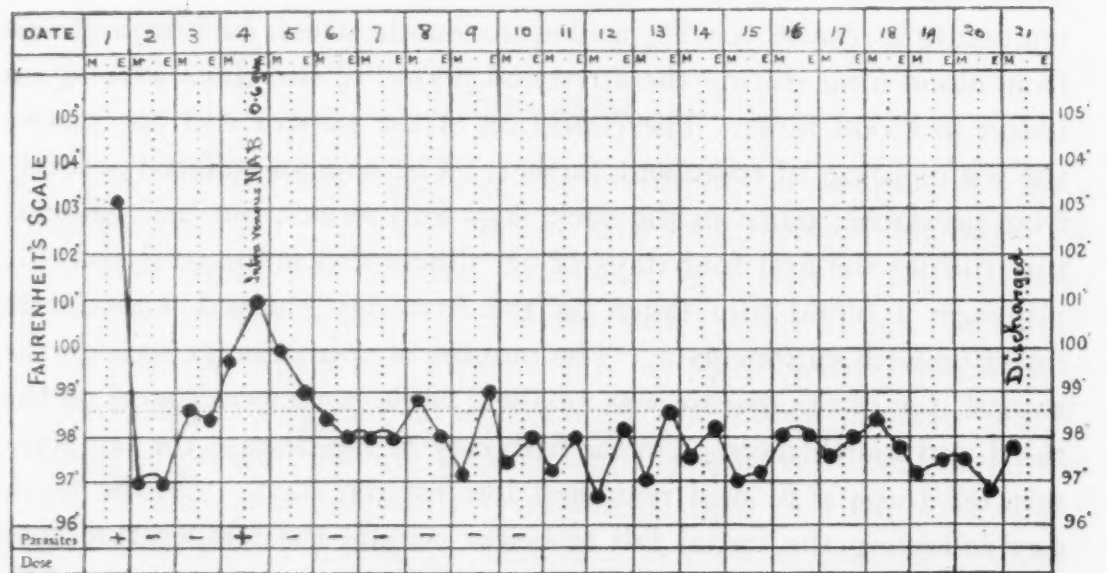
May, 1923

June



SEIDU SYIE, Male, Age 44. Relapsing Fever. Admitted June 1st.

June, 1923



treated in the Contagious Diseases Hospital. The following case is remarkable for its severity and for the unduly long period that elapsed between the patient's apparent cure and his relapse. The

patient, a well nourished young adult of 23, was admitted to hospital together with a large number of other cases, suspects and contacts, on 29th April, 1923, suffering from signs and symptoms of relapsing fever. On the 30th April his temperature was 105° F.

MUMUNI III, Male, Age 23. Relapsing Fever. Admitted May 2nd.

May, 1923

First attack.

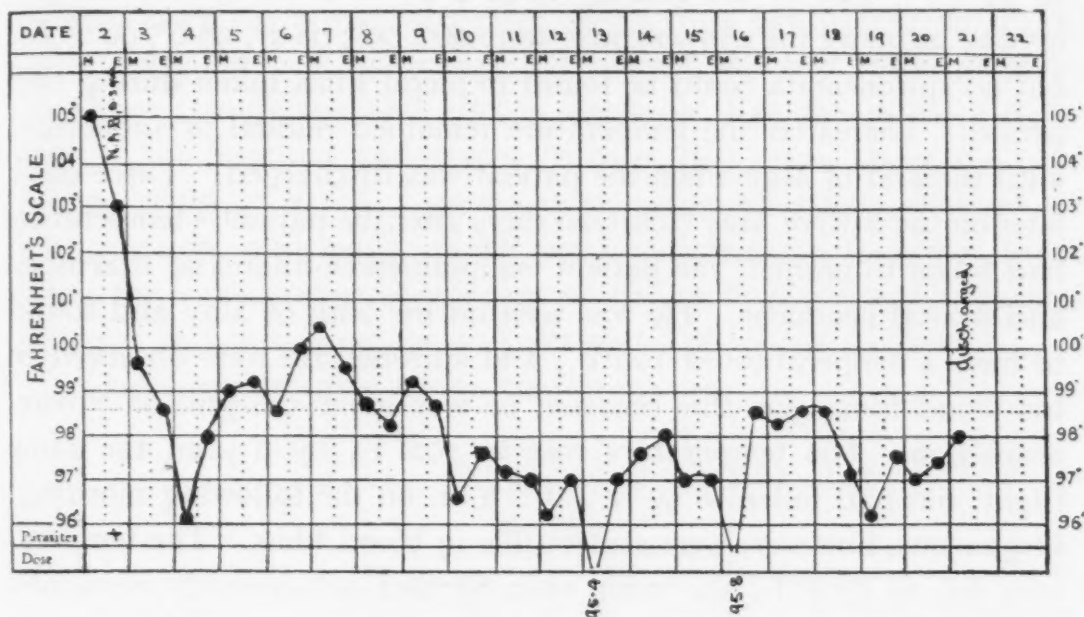


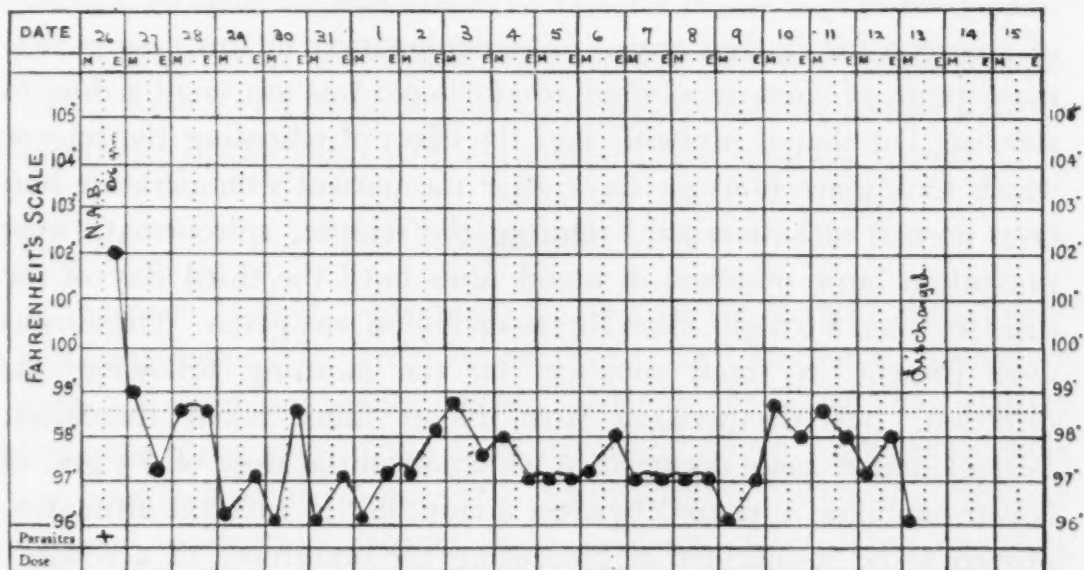
Chart to illustrate occurrence of relapse after an unusually prolonged period of apyrexia possibly resulting from the administration of an inadequate dose of Novarsenobillon during the first attack.

MUMUNI III, Male, Age 23. Relapsing Fever. Admitted May 26th.

### Relapse.

May, 1923

June





On the following morning his temperature was  $102.6^{\circ}\text{F}$ . but a blood film failed to show the presence of spironemata. His temperature on the following day was  $105^{\circ}\text{F}$ ., spironemata were present in a blood film, and he was given 0.3 gm. Novarsenobillon intravenously. By the evening of the same day the patient's temperature had fallen to  $103^{\circ}\text{F}$ . The next morning the temperature had still further fallen to  $99.6^{\circ}\text{F}$ ., and by the evening to  $98.4^{\circ}\text{F}$ . For the 3rd to the 9th of May inclusive the temperatures were  $96^{\circ}$ ,  $99^{\circ}$ ,  $98.4^{\circ}$ ,  $98.6^{\circ}$ ,  $99.2^{\circ}\text{F}$ ., but no spironemata could be found in blood films taken during this period. Thereafter the temperature remained normal or subnormal until the 21st of May when the patient was discharged. Three days later on the 24th of May (fourteen days after the patient's temperature had fallen to normal) the patient complained of anorexia, diarrhoea and frontal headache. He was seen on the 26th of May and found to have a temperature of  $100^{\circ}\text{F}$ ., and spironemata were observed in his blood film. On this occasion he was given 0.6 gm. of Novarsenobillon. His temperature rose to  $102^{\circ}\text{F}$ . by 6 p.m. the same night, but had fallen to  $99^{\circ}\text{F}$ . at 6 a.m. on the following morning. Organisms, however, were still visible in blood films. The temperature fell to  $97.4^{\circ}\text{F}$ . the same evening and subsequently remained normal or subnormal until the 13th June when he was discharged, his temperature not having been raised for sixteen days. The interesting point about this case is that the original pyrexial period lasted for about eleven days, followed by an apyrexial period of about fourteen days, when a relapse occurred. The pyrexial period during the relapse lasted for four days and was followed by a period of apyrexia for sixteen days. The explanation in this case is that the 0.3 gm. of Novarsenobillon administered was too small a dose to sterilise, but that it probably had the effect of retarding the relapse which took place fourteen days after the patient's temperature had been normal or subnormal. During the relapse, spironemata were present in large numbers in blood films until the third day of the disease, when a 0.6 gm. dose Novarsenobillon was given. Organisms were present in small numbers on the morning following the injection, but disappeared from blood films taken thereafter. Several similar cases occurred in which an initial dose of 0.3 gm. of Novarsenobillon appeared to effect a cure, but in which it ultimately proved to be insufficient in preventing the occurrence of a relapse.



A few patients relapsed even after receiving 0.6 gm. of the drug, but for routine work, dealing with a large group of persons, this dose appeared to be satisfactory, followed by 0.3 gm. or 0.6 gm. in the small number of cases failing to react to the initial dose, or showing signs of relapse.

Briefly, in untreated cases relapses occurred with an apyrexial period varying from two to ten days, while in treated or partially treated cases the apyrexial period varied from two to fourteen days. As a rule, but not invariably, the relapse was less severe both in signs and symptoms and also in duration than the initial attack. Out of the hundred and seventeen cases that came under the care of one of us (P. S. S-C.) twenty-three, or 19 per cent., relapsed on one occasion, and eight, or 6.8 per cent., relapsed a second time. Thus the total number of relapses in the one hundred and seventeen patients was thirty-one, or 26.5 per cent., of all the patients.

The following table shows the results:—

TABLE IV.

	Blood film		Unknown	Total
	Positive	Negative		
First Relapse ... ..	11	7	5	23
Second Relapse ... ..	3	5	...	8
Totals ... ..	14	12	5	31

In cases where the organisms could not be found in blood films, the diagnosis of relapse was based upon rise of temperature and the recurrence of the signs and symptoms of the original attack.

#### IMMUNITY

*Second Attacks.* Immunity is said to be of short duration in relapsing fever. It would be unfair to draw any such conclusions from the epidemic under review, since so little time has elapsed since the occurrence of the cases described above. One undoubted second attack occurred, however. The patient, a European, originally became accidentally infected with relapsing fever while injecting

infected blood into a rat. Seven days later he developed the signs and symptoms of the disease. At first no spironemata were seen in his blood, but after two days of moderately severe pyrexia the organism was found to be present in small numbers. The patient was treated with 0.3 gm. Novarsenobillon intravenously, and rapidly recovered. This first attack occurred in the beginning of April. At the end of June the same individual contracted a severe attack of the disease as the result of feeding infected lice on his forearms—two escaped and are thought to have been scratched into his skin. The second attack was rather less severe than the first, but reacted to treatment with Novarsenobillon as rapidly as had been the case in the first attack. If the patient acquired any immunity from the first attack, and this is probable, since he subsequently carried out a series of experiments, feeding on his forearms lice from relapsing fever patients, the immunity was of decidedly short duration, in fact less than eleven weeks.

#### TREATMENT

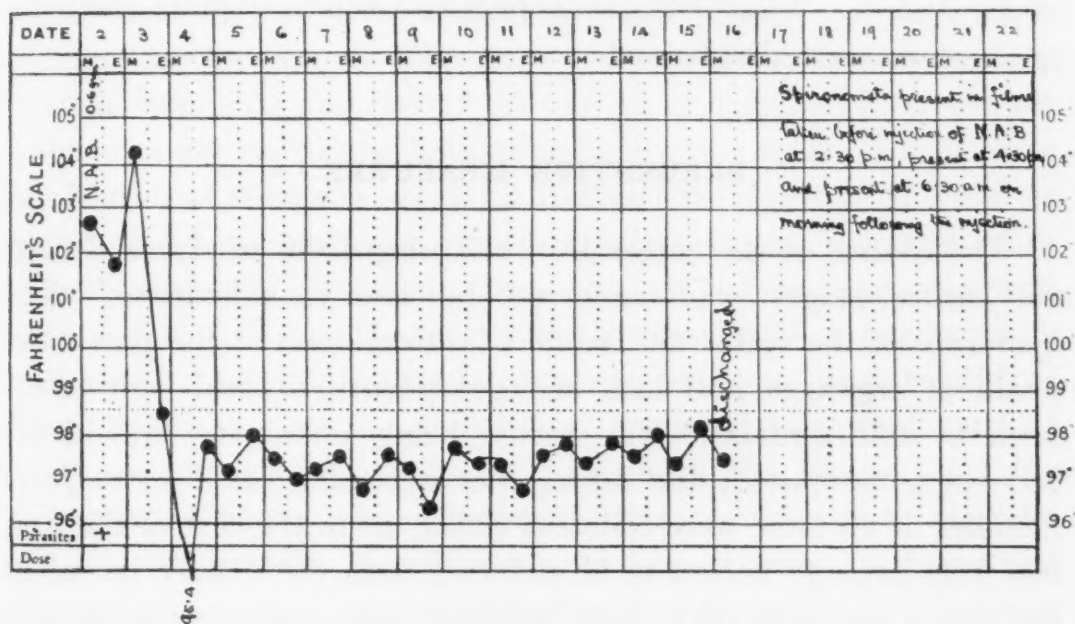
Apart from intravenous medication with Novarsenobillon given intravenously in 10 c.c. of warm, sterile, distilled water in doses varying from 0.3 gm. in the early cases to 0.6 and 0.9 or 1.2 gm. (divided into two to three doses) in the later cases, the treatment administered to patients was symptomatic. Patients were washed, shaved and disinfested on admission, and put to bed with a sleeping mat and two warm blankets. They received their injection of Novarsenobillon as far as possible on a fasting stomach. If they wished, they were given two biscuits and a drink of milk a short time after the injection. Cold sponging was resorted to where the temperature was 103° F. or higher, but otherwise, apart from being given as much water in small quantities as they wished, the patients were left to sleep quietly until the following morning, care being taken to avoid chills in cases where the temperature fell by crisis. The following morning a saline purge was administered, and if conditions were satisfactory and no vomiting was present a light diet was given. It was noticed that the patients not only wanted to get up and resume their usual everyday life immediately after the fall of temperature had occurred, but that they wished to resume a normal diet at once. As a rule the attack left the patients somewhat weak,

and vomiting immediately occurred, if the patients were allowed to satisfy their ravenous hunger. In those patients who reacted well to the treatment and in whom no elevation of temperature occurred within the fourteen days subsequent to the fall of temperature to normal, attempts were made as far as possible to graduate both diet and exercise until, for some days before the patients were due for discharge, they had resumed a normal life.

In order to obviate the possibility of relapses, patients were kept in hospital until their temperatures had been normal for fourteen days. Blood smears were taken of all patients with temperatures

BUKARI, Male, Age 38. Relapsing Fever. Admitted July 2nd.

July, 1923



Typical moderately severe case showing fall of temperature by crisis 24 hours after administration of 0.6 gm. Novarsenobillon.

above 99° F., and even if spironemata were not found in the blood films and other conditions could be excluded, further treatment with Novarsenobillon was carried out. This step appeared to be justified in the light of the subsequent histories of such cases. Among the one hundred and seventeen patients admitted to the Contagious Diseases Hospital, eighteen had already received an intravenous injection of 0.6 gm. Novarsenobillon. Injections—three of which were given intramuscularly owing to the inability to discover a vein of adequate size—to the number of one hundred and

thirty were given by one of us (P. S. S-C.). Of the total number of injections one hundred were of 0.3 gm. and forty-eight of 0.6 gm. Patients who received but one injection numbered one hundred and seventeen, those who received two numbered twenty-three, while eight patients had to receive a third injection. Generally speaking, apart from a little vomiting for two or three days after the injection (this vomiting occurred in untreated cases and consequently may have borne no relation to the intravenous medication) no ill-effects were experienced from the use of Novarsenobillon. One death occurred in a series of one hundred and seventeen cases that came under the notice of one of us (P. S. S-C.), but the patient was in a serious condition prior to the injection, and when death occurred on the third day following the injection, it could be attributed with fairness to the disease. West Africans appear to tolerate organic arsenical preparations exceedingly well.

#### PREVENTIVE MEASURES

The control of the outbreak of relapsing fever in Accra during the earlier stages was hampered by two considerations, viz., ignorance of the vector and mode of transmission of the particular strain of organism and lack of legal powers to deal with cases, suspects and contacts. The vector being unknown, lice, ticks, bed bugs, mosquitoes and biting flies were all treated as suspect. Samples of all these were collected either, as in the case of the first two from patients known to be suffering from the disease or, as in the case of the remainder, from bedding, mats and other articles in infected premises, and were taken to the Medical Research Institute.

Measures were taken against all possible vectors, and with this end in view attention was concentrated on premises situated in congested areas of the town occupied by Hausas and members of Northern Territory tribes, whose habits in regard to overcrowding and a marked aversion from cleanliness, adequate lighting and ventilation were well known. Careful house-to-house visits were made in those areas and throughout the town, as many as 46,013 being carried out during March, April, May and June, one of us (P. S. S-C.) being responsible for 1,006. During these inspections personal, domestic and general cleanliness was preached, all old



sacking, lousy bedding and other refuse being removed from compounds. Moreover, efforts were made to see that all houses were provided with adequate lighting and ventilation. During these visits a practice was made of urging any person who appeared to be ill and suffering from fever to go for treatment to the Native Hospital.

The procedure adopted early in the epidemic when a case of relapsing fever was reported was for the patient to be admitted into the Native Hospital, for his quarters to be disinfected and disinfested and for a watch to be kept on all contacts, any of whom showing signs of fever being urged to report to the Medical Officer at the Native Hospital.

By the fourth week of the outbreak it was evident that infection which appeared to have been introduced into Accra from bush villages was spreading to other parts of the town from houses already infected with relapsing fever. To obviate this tendency to spread, it was strongly urged that legislation should be passed, in order that the Health Authorities might round-up all persons suffering or suspected to be suffering from relapsing fever, together with contacts with such cases, for the purpose of segregating them and sterilising them as far as concerned the presence of spirochaeta in their blood. Legislation was not passed, however, until the 28th of April, by which time the disease had appeared in several parts of the town, though principally in Tudu and the Zongo. On the 29th of April, acting within the powers obtained through this legislation, it was possible to effect a round-up of cases, suspects and contacts on a much larger scale, so that within twenty-four hours of legislation being passed, over three hundred and sixty-three patients, suspects and contacts were removed from infected premises to the Contagious Diseases Hospital. Where necessary, one or two contacts were allowed to remain in such infected premises, to safeguard the property from thieves, to keep the premises clean and to care for any horses or other animals. Such persons were visited daily to exclude the possibility of their having contracted the disease.

The health of the person permitting, a routine was adopted at the Contagious Diseases Hospital in almost every case. On admission all male cases, suspects and contacts had their heads,



armpits, beards and pubic hair shaved, while in the case of females the hair was close clipped and shaved from their armpits and pubes under the supervision of a female sanitary inspector. Subsequently the shaving was followed, where physical conditions permitted, by a sea bath and by a wash-down where a sea bath was inadvisable. All clothing was shed into barrels containing a 5 per cent. solution of Izal prior to the bath being taken, and after the bath a warm blanket, sleeping mat, and cup and plates were issued to everyone. Temperatures and blood smears were then taken and recorded and the groups dealt with were allotted accommodation in three classes of huts, according as to whether they were thought to be suffering from relapsing fever or were merely suspects, or contacts with cases and suspects. Special diet, as for example, milk, tea, broths, etc., was given to patients, whilst the remainder received two meals per day. Hot Akasa was given in the early morning as soon as temperatures had been taken, and was followed at mid-day by a large meal of rice, plantain, fula or other foodstuffs purchased in the markets. As far as possible the tastes and wishes of patients and contacts were consulted with regard to the variety of food supplied. Contacts were detained for fourteen days, during which time they were given a certain amount of work to do in the way of scrubbing out huts, keeping the segregation compounds clean, helping with the chopping-up of firewood, with the preparation of food and drawing of water. They enjoyed sea baths daily, arrangements being made for the opposite sexes to bathe at different times. At the end of the quarantine period, if their temperatures which were taken morning and evening had remained normal, the contacts were again submitted to a thorough shaving, and were given a bath, and then had their disinfected and disinfested clothing returned to them and thereafter were discharged. Contacts or suspects who developed raised temperatures, or in whom blood films proved to be positive as regards the presence of spirochetes, were immediately transferred to the huts reserved for patients. After receiving appropriate medication, patients were kept in hospital until they had been free from pyrexia for fourteen days—blood films being taken daily while temperatures were raised.

It is noteworthy that the following method of disinfecting and disinfesting clothes and blankets appeared to give the best results.

The articles to be disinfested were first soaked for forty-eight hours in barrels containing 5 per cent. solution of Izal. They were then washed and placed in the sun during the middle of the day on sheets of corrugated iron. This resulted in most efficient disinfestation, for the heat generated was at least 150° F. Neither lice nor eggs capable of hatching survived this treatment. Purses and amulets, the latter carried in great numbers by Hausas and Northern Territory tribesmen, required special treatment.

In order to minimise the risk of infection being carried from the hospital to Accra, the auxiliary staff of the hospital were persuaded to stay in special quarters reserved for them in the grounds of the hospital, and all the staff, including one of us (P. S. S.-C.), took further precautions by frequent baths and by shaving the hair from axillae and pubes. A police guard was maintained at the hospital during the period of the outbreak, and the Non-Commissioned Officer in charge is to be congratulated in not losing a single patient or contact.

### CONCLUSIONS

1. This first recorded outbreak of relapsing fever in British West Africa is due to a spirochete conveyed by lice.
2. As regards inoculation experiments, monkeys, black and white rats become infected with the strain, but do not relapse; guinea-pigs and rabbits are refractory, whilst the pouched rat becomes infected and relapses.
3. The vectors of the organism in the present epidemic and the inoculation experiments suggest that the parasite is not the *Sp. duttoni*, but corresponds more closely to *Sp. recurrentis* (vel *obermeieri*), or a related strain.
4. Novarsenobillon is a specific in the treatment of the disease.
5. Immunity does not appear to be lasting or complete in cases treated with Novarsenobillon.

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## CARBON TETRACHLORIDE IN FILARIASIS

BY  
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*(Received for publication 23 August, 1923)*

In view of the successful treatment of intestinal nematodes with carbon tetrachloride administered orally, it was decided to try the effect of the drug administered intravenously and intramuscularly on filariasis.

Prior to treating infected human beings, a series of experiments were carried out on healthy dogs. It was found that dogs could stand relatively large amounts of the pure drug administered intramuscularly or intraperitoneally, and that older animals tolerated the drug better than young animals; the minimal lethal dose was found to be 0.25 ccs. (*i.e.*, 0.39 gms.) per kilo body weight in animals weighing less than 1 kilo, whereas animals weighing over 4 kilos showed symptoms from which they recovered after a dose of 0.6 ccs. (*i.e.*, 0.94 gms.) per kilo body weight; these symptoms were drowsiness and refusal to take food for a few days after the injection.

Injected intravenously the drug caused rapid death due to embolism, but when mixed with two parts of ether by volume, embolism after intravenous injection was avoided.

An intramuscular injection of 0.5 ccs. of the drug into a healthy human being caused irritation at the site of injection, which was not severe and passed away in a few minutes; shortly after the injection the distinctive taste of the drug was felt in the mouth.

After these preliminary experiments the drug was tried on four adult patients each with a slight infection of *Filaria bancrofti*, as judged from the number of microfilaria in the circulating blood. In each case the number of microfilaria per c.c. was estimated by counting the number of microfilaria in 20 cmms. of blood at 9 p.m., before commencing treatment and at the end of treatment.



CASE 1. A native aged 35, weight 144 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—50 per c.c.

25.6.23. Intramuscular injection of 0.5 c.c. carbon tetrachloride.

20.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 1.5 c.c. carbon tetrachloride.

30.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

CASE 2. A native aged 32, weight 122 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—100 per c.c.

20.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 1.5 c.cs. carbon tetrachloride.

CASE 3. A Native aged 25, weight 143 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—350 per c.c.

11.7.23. Intramuscular injection of 1.1 c.cs. of carbon tetrachloride.

16.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 1.5 c.cs. carbon tetrachloride.

30.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

CASE 4. A native aged 24, weight 154 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—100 per c.c.

20.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

30.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

All the intramuscular injections were given into the buttock.

The patients were observed for one hour after each injection.

The intramuscular injection caused local pain, which passed away in from five to ten minutes. No marked analgesic effects were noticed after the local pain had disappeared. Injection of even 2 ccs. of the drug intramuscularly produced no anaesthesia.

Generally there was a slight diminution in the pulse rate up to four beats per minute following intramuscular injection.

All the cases noticed the taste of the drug shortly after injection.

Intravenous injection of the drug mixed with ether caused a severe attack of coughing, which commenced during the injection and lasted a few minutes; one case complained of a burning sensation in the mouth; all the cases were sleepy after the intravenous injection,



but whether the sleepiness was caused by the ether or the carbon tetrachloride it is impossible to say.

None of the cases showed albuminuria or other ill-effects either during or after the treatment, which was abandoned after the 30th July, 1923.

None of the cases showed any marked diminution of the microfilaria in the blood after the treatment, but it is impossible in the case of *Filaria bancrofti* to form an opinion of the effect of the drug on the adult worms.

The action of intravenous or intramuscular injections of carbon tetrachloride on adult filaria can only be tested in cases of *Loa loa*, but up to the present no suitable cases have been found in Freetown.

In view of the comparative safety with which the drug can be administered, both intravenously and intramuscularly, it is hoped that it will be tried on cases of *Loa loa* in localities where that disease is common.

I have to thank Dr. J. Y. Wood, of the W.A.M.S., for the opportunity of carrying out the above treatment, and Dr. P. A. Maplestone, from whose series of routine examinations for parasites the cases were selected.



# YELLOW FEVER IN THE GOLD COAST: ITS ENDEMIC AND EPIDEMIC CHARACTER

BY

R. O. WHITE

WEST AFRICAN MEDICAL SERVICE

*(Received for publication 24 August, 1923)*

The recently reported cases of yellow fever in different parts of the Gold Coast\* bring into prominence again the subject of its endemicity in West Africa, and suggest a consideration of the conditions which give rise to these periodic outbreaks.

That the West Coast of Africa has been an endemic centre no one disputes, but opinion is divided upon the question of the presence of yellow fever as an endemic disease to-day.

The Yellow Fever Commission (West Africa) appointed by the Colonial Office in 1913 considered the evidence submitted was in favour of the belief that West Africa is an endemic centre (1916). More recently, Guiteras (1921) and Hoffman (1921) expressed the opinion that this centre has already ceased to exist. This supports the view held by Ross, who suggests that the *Stegomyia* mosquito is not sufficiently numerous to permit the disease to maintain itself.

The investigations of the Rockefeller Foundation in 1920 failed to discover a single case, and we find that out of nearly half a million recorded illnesses treated in Nigeria during the years 1919-21, there is but a single diagnosis of yellow fever.

In spite of this formidable array of opinion and facts, it is not possible to ignore the significance of the existence of a disease which does not conform to prevalent types of fever and which is capable of being diagnosed as yellow fever.

The reports of the recent cases occurring among Europeans on the Gold Coast leave very little room for doubt as to the accuracy of the diagnosis. In all these were the black tarry or coffee grounds vomit, a marked diminution in the quantity of urine—amounting in

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\* A cable received at the Colonial Office on July 11, 1923, stated that 25 cases of yellow fever had occurred in the Gold Coast since November 1, 1922—18 in Europeans, all fatal, and 7 in natives, 2 fatal.

some cases to complete anuria—with albuminuria, associated with symptoms of extreme urgency followed rapidly by death. When post-mortem examinations were made, fatty degeneration changes were found in the liver and kidney. These features were common to all, but haemorrhage from mucous membranes, conjunctival injection, varying degrees of jaundice, Faget's sign and high fever are described. It is also noteworthy that, with one exception, the fatal termination occurred within a week of the onset of the illness.

In the face of such evidence, it must be admitted that yellow fever is a disease of West Africa. The next important point is—in what form does it exist?

An examination of the trade routes between the West Coast of Africa and other parts of the world fail to supply us with an external source of infection. In the 1911 outbreak at Bathurst it was thought that the occurrence of the disease at this port was connected with the visit of the s.s. 'Akassa.' Subsequent enquiries, however, failed to confirm this. In the epidemic under consideration, there is not the slightest ground for suspecting that the disease was introduced from without. It seems, then, that we must assume that yellow fever does exist in West Africa, in some latent form requiring special conditions for its development.

That special conditions are required may be gathered from the infrequency with which the disease occurs among Europeans in any one place, notwithstanding that the essentials for the maintenance and spread of the disease are always present—the virus, the carrier and the non-immune.

The explanation which used to be given for the comparative freedom from yellow fever among Europeans on the West Coast of Africa was, that the newcomer usually became the subject of a mild infection which conferred immunity for the remainder of his stay in the country. But this contention cannot be maintained, in view of the fact that victims of the disease have succumbed to it after periods of residence up to thirty years. It is necessary, therefore, to look for some other explanation.

When an endeavour is being made to discover the aetiological factor concerned in the outbreak of an infectious disease, it is helpful to be able to note the conditions under which it has died out in certain places.

Peterson's (1922) view of the spontaneous elimination of yellow fever in St. Thomas—which is based upon Carter's principle of 'the failure of the human host'—suggests that the virus gradually becomes attenuated until it reaches the point of extinction. In other words, the commerce between the mosquito and the unresponsive native eradicates the disease, not by the process of conferring general immunity, but by the death of the virus. The logical inference which may be drawn from this is, that the West Coast of Africa would cease to be a yellow fever area as soon as, or shortly after, less immune individuals were excluded from the country. The European is, of course, the obvious non-immune. Yet if we study the records of yellow fever epidemics, we shall find long intervals between outbreaks in any one place, and that they are traceable to a non-European source. This is fair presumptive evidence that the European is an accidental victim rather than an agent in maintaining the disease.

It may be urged that mild cases occur among Europeans which escape diagnosis. The mortality rate in the 1910-11 outbreaks (1913), which show six recoveries in forty cases, is against this; more especially when the reports of these recovered cases are scrutinised.

Of the six, three are included without any details of their illness, and as such, merely indicate people who were sick at the time.

Of the three remaining, two are so untypical of yellow fever, that it is safe to presume their inclusion was due to the prevalence of the disease rather than to the character of their physical signs and symptoms. The last case of this group appears to have responded, eventually, to energetic treatment with quinine. Albuminuria was absent throughout the illness.

In the present epidemic in the Gold Coast, the mortality among the Europeans attacked is 100 per cent. If we exclude the six doubtful cases referred to above, it would appear that yellow fever in West Africa is invariably fatal to Europeans. At any rate, it may be said it shows itself in a way which is not likely to be overlooked.

To recapitulate: The indigenous native, dissociated from the presence of newcomers, is incapable of maintaining the virus. The disease is not overlooked when it occurs among Europeans. The intervals which elapse between outbreaks among Europeans are proof



that the European is not responsible for the fact that yellow fever is an endemic disease in West Africa.

It is obvious then that some other section of the community keeps the virus alive in a 'larval' state.

It has been said that previous recorded outbreaks are traceable to a native source. In the Reports of the 1910-11 epidemic (1913) it will be found that in every case when mention is made of living conditions, there is the association of close native proximity to the European attacked. Further it will be found that the native element is an imported one, either from the confines of the colony itself, or from a more remote part of West Africa.

Cases numbers 43 and 44 were Kroo-boys. 37 was a Yoruba lately come to Lagos, where he was taken ill. 29 and 38 also occurred in Kroo-boys. 28 was a Hausa. 19 and 20 were of the Mendi tribe living in Freetown at the time they were taken ill.

The foregoing comprise all these cases among natives where it is possible to identify their nationality. The remainder come under the, not very illuminating, description 'A native born and bred in West Africa.'

In the Gold Coast epidemic of this year, the cases at Saltpond were traceable to a Kroo-boy who died of yellow fever shortly after his arrival there. Later, eighteen miles away, Cape Coast was attacked, isolated cases subsequently occurring at Winneba, Accra, Keta and Secondee.

On the Gold Coast we find that West Africans who come to the Colony pursue one of the following callings:—Trading, soldiering, mining, as railway and road labourers, or manual work at the seaports. With the exception of the last-named class, it will be found that the principle of segregation is conformed with. The migratory trader, the Hausa, has his Zonga to go to, the soldier his barracks, while railway and road labourers (usually drawn from the Northern Territories) have carefully supervised camps.

The porter of the Gold Coast, the Kroo-boy, has no such provision made for him, and he is to be found, as a rule, domiciled in the compound of his employer, usually a European. He also has access to the native quarters of the town, and consorts with other Kroo boys who are employed by native merchants. There is here, then, a connecting link between the two classes which have been

excluded as not being responsible for maintaining the disease. The Kroo-boys also represent, numerically, a section of the community which must be taken into consideration, and they become suspects, partly because the process of elimination adopted here has left them unexonerated, and also for the reason that they are known to contract the disease and to have been responsible for outbreaks among Europeans.

The question now arises, how is it that these Kroo-boys are more susceptible to infection than the indigenous native?

It will be shown later that the immunity to yellow fever with which all West Africans are endowed is merely relative in degree and breaks down under certain conditions. It is suggested here that one such condition is change of environment.

Apparently the native's degree of immunity is sufficient so long as he remains in his own country, but becomes impaired when he goes to live in another part of West Africa. There is support for this assumption in the fact that West Africans do contract 'fever' when they leave one part of West Africa to take up residence in another. This has been recognised by Government Medical Officers, and the West Africans themselves are aware of it. An opportunity of observing this phenomenon occurs when a native official is transferred to a new station, or is on leave from a Colony of which he is not a native. That the 'fever' mentioned above is often due to malaria, there is very little doubt. An intensive and fatal case of this disease was seen at Accra in a Kroo-boy who had been resident in the Colony for six months. It is known that malaria does not attain to such severity in the adult indigenous population of West Africa. The influence of environment is, therefore, a factor which must be reckoned with when the subject of immunity is being considered.

From these premises it is easy to reconstruct the sequence of events. The Kroo-boy who migrates to some other part of West Africa automatically becomes more susceptible to yellow fever. He contracts the disease in a mild form, unrecognisable as such, and causing very little, if any, inconvenience. But an impetus has been given to the virus which, under favourable conditions, ultimately becomes so enhanced as to give rise to definite illness. The final stage is reached when living conditions make it possible for the

Kroo-boy to pass on this infection to a European. When this occurs the virus has attained to a very virulent degree of toxicity, which if unchecked by the wholesale destruction of the mosquito, will be capable, ultimately, of infecting—sometimes with fatal consequences—the indigenous and erstwhile unsusceptible native. This happened both at Saltpond and Cape Coast during the present epidemic, and is proof of what has already been said, that the West African's immunity is merely a relative one.

It is well known that any break in the chain of essentials which go to produce a yellow fever infection is sufficient to stop, or at least interrupt, the process. The lapse of time which takes place between observed epidemics in West Africa, seems to suggest that the chain is delicate in its construction and that the process of building up the virus sufficiently to produce recognisable effects is a long one. Segregation of Europeans—in as far as it obtains in West Africa—appears to have the effect of lengthening the process. It has certainly provided immunity for the segregated, for in no single instance has a case occurred among them. When it is remembered that in segregation areas native servants—often Kroo-boys and natives of the Northern Territories—live in close contact with the European, it would seem that the slightest precautions are sufficient to prevent infection. As Carter suggests in his statement of requirements for the maintenance of a yellow fever infection, the number of mosquitoes may fall short of what is necessary. It probably will be found also, that non-interference with the mosquitoes, overcrowding and lack of light and ventilation are necessary. Routine sanitary work probably intereferes from time to time with one or other of these subsidiary requirements, and has the effect of delaying the development of the virus. But sooner or later, it would seem, an area escapes over a period which permits it to become intensely infective, and an outbreak of yellow fever results.

The localised character of these outbreaks in a town is due to the well-marked domestic habits of the mosquito concerned. If we look at the spot maps accompanying the Reports of the 1910-11 epidemic, it is easy to see the human agency which carries the disease from one part of a town to another, over distances which leave intermediate areas unattacked.

It is, therefore, the infected rather than the infective element

which is responsible for the spread of the disease. If we can control the former and keep it from coming into close living contact with the unsegregated European, there is a reasonable prospect of preventing re-occurrences of these outbreaks. Efforts at controlling the other element have hitherto met with very little appreciable success. That temporary success is obtainable has been amply demonstrated during the present epidemic. Towns where the *Stegomyia* index is normally 80 per cent. have, after a week's intensive work, had this figure reduced to below 5 per cent. The means employed, other than fumigation of the area in which cases occurred, were the usual mosquito brigades under the supervision of European volunteers.

The effectiveness of this measure, when considered in the light of what has already been said, suggests the advisability of instituting a 'cleaning-up week' at least once a year, in every town where cases of yellow fever have been known to occur within the last twenty years. It should also be a matter of routine that when a case of yellow fever is reported in a Colony, every town with which the infected area is connected by road, rail, or sea, should immediately start energetic anti-stegomyia measures. This will prevent outbreaks elsewhere, for the reason that the number of mosquitoes remaining will not be able to maintain the disease. At any rate, the possibility of a secondary focus being established will be a very remote one. In the intervals between epidemics, Government Medical Officers and other Medical practitioners should be asked to observe carefully cases of fever which occur in West Africans who are strangers in the place, with a view to early diagnosis, thus ensuring prevention of the development and spread of the disease.

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## MISCELLANEA\*

### KURLOFF BODIES IN FISH

In a specimen of *Diodon hystrix* examined at Freetown, Sierra Leone, Kurloff bodies were found in about 70 per cent. of the lymphocytes. As many as five Kurloff bodies were present in some cells.

S. ADLER and E. J. CLARKE.

### TRICHONEMA TETRACANTHUM (MEHLIS, 1831, OF LOOSS, 1900)

This worm was found by us in June, 1923, in a donkey born and bred in the north of Ireland. This record is of interest, as the parasite has not been found since it was described by Looss in 1900. The fact that it had not been observed in Europe is used by Railliet (1923) as an argument that Looss' parasite is not identical with *Strongylus tetracanthus*, Mehlis, 1831.

J. W. S. MACFIE and WARRINGTON YORKE.

### PIGS AND ANKYLOSTOMIASIS IN THE GOLD COAST

During January and February, 1922, forty-eight pigs were examined at the Accra slaughter-house for hookworms, but neither *Ancylostoma duodenale* nor *Necator americanus* were found, although 3,270 other small nematodes were collected, the majority of them being *Oesophagostomum dentatum* (Rud., 1803), and a few

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\* It is proposed to publish under this heading short records relating to Tropical Medicine and Parasitology.

*Arduenna strongylina* (Rud., 1819) and *Characostomum longemucronatum* (Molin, 1861). Subsequently specimens obtained from pigs at Cape Coast, at Kumasi, and at Sekondi (Dr. J. F. Corson) were examined, and in these also neither *A. duodenale* nor *N. americanus* was found. These results do not, therefore, support the view that pigs are an important factor in the dissemination of hookworm infections in the Gold Coast.

J. W. S. MACFIE.

### ONCHOCERCA ARMILLATA IN CATTLE IN THE GOLD COAST

Commes and Devanelle (1917) record that in Upper Senegal and Niger, *Onchocerca armillata*, Railliet and Henry, 1909, is a common parasite of cattle, and that they found it in one hundred and fifty-one animals out of one hundred and ninety-eight, that is in 76.3 per cent. It is also a common parasite of cattle in the Gold Coast, particularly of the hump-backed breed, as is shown by the fact that of forty animals examined at Accra during September and October, 1922, namely, sixteen hump-backed cattle and twenty-four of the straight-backed breed, fourteen, equal to 87.5 per cent., of the former, and seven, equal to 29.2 per cent., of the latter, were infected.

The situations in which the worms were found and the lesions (atheroma, calcification, cyst and nodule formation, etc.) associated with them were similar in the Gold Coast cases to those described by Commes and Devanelle, and need not be referred to in detail. Some of the nodules contained, in addition to a mass of fibrous material and portions of parent worm, a number of free larvae. The larvae resembled in general form those of *O. volvulus*, length of the few measured 280 $\mu$  to 345 $\mu$ , breadth about 5 $\mu$ , anterior end rounded, nerve ring well marked and situated at about 25 per cent. of the length from the anterior extremity, and tail sharply pointed.

In three infected animals, the blood (10 c.c. or more) was examined for larvae, but without success. In this connection it may be recalled that in blood films from one hundred and sixty-six cattle examined at Accra in 1914, filarial embryos were found in five,

that all these were sheathed and were perhaps embryos of *Setaria labiato-papillosa*, a species which has been found in cattle at Accra (Macfie, 1915), but that no larvae resembling those of *O. armillata* were encountered. The skin of these three animals was also examined, because it was thought that, as in the case of *O. volvulus*, larvae might be present in it. No larvae were found, but it must be admitted that considerable difficulty was experienced owing to the thickness, density, and hairiness of the skin, and that consequently the examination was not a very satisfactory one.

J. W. S. MACFIE.

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LIVER  
OF THE  
MEDICAL



ANNALS  
OF THE  
TROPICAL  
MEDICAL  
AND  
HYGIENE  
PUBLISHED BY  
THE LANCET  
LONDON  
AND  
NEW YORK

# Tropical Medicine

AND  
TROPICAL  
HYGIENE

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NEW YORK





## THE MODERN TREATMENT

The value of inorganic acids of the Chlorine group alone or in combination—in the treatment of leprosy—has been clearly demonstrated from leprosy, which is the

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# MOORE

Mixture of Esters of  
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of leprosy, and the results  
from 1900 to the present  
are available for the doctor

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SURROGATE

# A STUDY OF THE TUMBU-FLY, *CORDYLOBIA ANTHROPOPHAGA* GRÜNBERG, IN SIERRA LEONE

BY  
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AND  
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(Received for publication 15 August, 1923)

## PLATES XV—XVIII

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## I. INTRODUCTION

Although it is more than sixty years since human Myiasis due to the larva of *C. anthropophaga* was first investigated by two French naval surgeons in Senegal, it is a remarkable fact that even to-day complete unanimity as to the mode of infection, the seasonal incidence, the natural reservoirs of the infection, and the appropriate prophylaxis, is by no means attained. It is interesting to find, in respect to this fly and also the South American fly, *Dermatobia cyaniventris*, that a disregard of native accounts has led research into side tracks which have been followed for long periods, before it was discovered that they were leading in the wrong direction. Nothing can better illustrate the manner in which this has occurred than the following extract from the excellent account of Cordylobia Myiasis given by Coquerel and Mondière (1862), who published a paper—the first so far as is known at present—on this subject. Writing of this form of Myiasis as observed by them at Portudal in Senegal, they say 'Cette singulière affection est connue des indigènes, qui savent très bien extraire les larves qui les tourmentent et viennent souvent se loger dans les tissus du scrotum de ces malheureux. Ils prétendent que ces vers sont produits par une petite mouche très commune à Portudal. Cette mouche pondrait ses œufs dans le sable humide, le ver y séjournerait jusqu'au moment où profitant du repos d'un homme étendu sur le sol, il s'introduirait dans la peau de sa victime.'

Unfortunately the authors were so little impressed by the probability of this suggestion that they added 'Il n'est pas besoin d'insister sur les détails de ce récit pour en signaler les erreurs. Il est évident que les larves du Diptère du Sénégal ont été déposés dans la peau, ou que les œufs ont été fixés à quelque poil de cette membrane dès leur origine et que les vers ne peuvent vivre ailleurs.' The theory held by natives in Senegal in those days with regard to the mode of infection by the larvae of Cordylobia, is the same which is held to-day by natives of Sierra Leone, and it is the theory which has been proved to be, in all essentials, correct, by the accumulated research of European observers up to the present day.

In a similar way we find that the work which has been done on the bionomics of *D. cyaniventris* in South America has revealed the

interesting fact that the eggs of this species may be transported on the body of a mosquito and deposited on the animal which is to serve as the host of the larva. It has taken many years to produce the scientific evidence that such a remarkable means of infection can occur, yet the native peasants have known it for so long that their name for the larva is *Gusano de Zancudo*, *i.e.*, the worm of the mosquito. The suggestion of such a means of infection was treated with frank incredulity by such observers as Da Silva Arango, who refers to the peasants' belief in the transference of *Dermatobia* infection by mosquitoes as 'a popular error, very widely spread throughout Brazil.' This incredulity of those who first investigated the bionomics of *Dermatobia* had the same effect as that mentioned above, in delaying the discovery of the fact that the mosquito, *Janthinosoma lutzi*, actually does transport the eggs of *Dermatobia*.

The suggestion has been made by Zepeda (1913) that the larvae of *Cordylobia* may be carried in the same way by mosquitoes; we have found no evidence of this.

The accurate knowledge displayed by the intelligent African native, the uneducated native who uses his powers of observation to the fullest extent, is often impressive. The Protectorate natives of Sierra Leone quite commonly make for example the nicest distinction between the bionomics of the testaceous flies, *C. anthropophaga* and *A. luteola*. They recognise that the flies are very similar to each other in appearance, and that each produces eggs out of which larvae proceed; they are perfectly aware, however, that of one, the larva lives in the tissues of the host and produces boils, while of the other, the larva merely feeds on the cutaneous blood of the host and lives in the ground, only emerging at night. They can also procure samples of either larva in a short time if required to do so, the former expressed from the larval tumours in the skin of affected animals, the latter obtained from the earth on the floor of their huts by the simple procedure of sleeping on a mat on the floor and searching under the mat in the early hours of the morning. It is, therefore, no more than justice to recognise how in Myiasis, as in very many other diseases, the knowledge of the natives, acquired by the slow and painful process of racial and personal experience, has assisted the investigator to the correct solution of the problems of disease.



## II. NOMENCLATURE

From the earliest days of our knowledge of Myiasis due to *Cordylobia*, that is to say, from the time of the publication of the communication of Coquerel and Mondière in 1862 until the most recent times, when in 1914 Roubaud published his well-known treatise on Myiasis in French West Africa, very various opinions have been held as to the probable relationship which existed between larvae from diverse hosts in different parts of Africa. Coquerel and Mondière, who were not fortunate enough to rear the adult fly, considered the larva to be that of an Oestrid.

Béranger-Féraud (1872) was successful in rearing flies which were identified by Émile Blanchard as belonging to the genus *Ochromyia*, Macq.; Blanchard called them by the new specific name, *Ochromyia anthropophaga*. Of this name Austen (1907) says, 'Since, however, no description of the fly whatever was given, *Ochromyia anthropophaga*, Émile Blanchard, is a mere *nomen nudum*, and consequently invalid.' The association of the name *Bengalia depressa* in 1891 with larvae from a human case of Myiasis has proved an additional complication, and still remains so to-day in some publications, in spite of Grünberg's work. Austen, in the paper mentioned above, gives a statement of how the larvae of human Myiasis due to *Cordylobia* came to be associated erroneously with the adult fly *Bengalia depressa*, Walk. He points out that the type of *B. depressa* is in the British Museum, and that although it is an allied, nevertheless it is a very different, insect from *Cordylobia*; he says, moreover, that the life history of *B. depressa* is as yet unknown, and that there is not a particle of evidence to prove that its larva is a subcutaneous parasite.

It was Grünberg (1903) who, after a careful examination of all the material available, both larval and adult, came to the conclusion that the fly did not belong to the genus *Ochromyia*, nor yet to *Bengalia* nor *Auchmeromyia*; but was a fly which required for its classification a new genus; he accordingly erected the genus *Cordylobia*, with the species *C. anthropophaga* (Blanch.). He gave a long and detailed description of genus and species. Dönitz (1905), in an article entitled 'Über eine neue afrikanische Fliege mit parasitisch in der Haut von Ratten lebenden Larven,' gives a description of what he considers to be a distinct species of



Cordylobia, and names it *C. murium*. At the same time, Dönitz reviewed the position with special attention to the ideas expressed by Grünberg and Gedoelst (1905), and shows how the latter came to speak of a *Cordylobia anthropophaga*, Grünberg. Dönitz himself proposed the name *Cordylobia grünbergi* for the East African form.

Roubaud (1914) gives reasons for deciding that *Cordylobia murium* should not be retained as a separate species, namely, that the differences claimed by Dönitz to exist are not sufficiently striking or constant to warrant the species; at the same time the name *Cordylobia grünbergi* is dismissed by him as invalid.

Two other species also are dealt with by Roubaud; of *Cordylobia praegrans*, Austen, he says that the subsequent discovery of the male has shown that it does not belong to the genus *Cordylobia* but should be placed in the genus *Chaeromyia*, Roubaud, while *Cordylobia rodhaini* should likewise be placed in another genus. To sum up, Roubaud says there appears to be only one species of Calliphorines belonging to the genus *Cordylobia* as defined by Grünberg; that is the fly of Cayor which was first bred by Béranger-Féraud in Senegal, *Cordylobia anthropophaga*, Blanchard. Austen, however, in his summary of the situation, referred to above, ends with the remark, 'The correct designation of this highly important and much misunderstood African Muscid is, therefore, *Cordylobia anthropophaga*, Grünberg. This authoritative statement we have, therefore, accepted.

### III. GEOGRAPHICAL DISTRIBUTION

Grünberg (1903) gave as the distribution of *C. anthropophaga* a list of places, which included Senegal, South-West Africa, Gaboon, Dar-es-salam, Zambesi, Lake Nyasa, Tanga, Delagoa Bay, Bagamoyo and Durban. The distribution later given by Roubaud is from Senegal and Lake Chad to the Cape. In the map included in his work, practically the whole region of Africa, south of about 16° North latitude, is shown as infected, with the exception of the North-East area. He points out, however, that the fly is irregularly distributed and that many large areas so far appear to be free of it, or that it has not been recorded from them.

OCCURRENCE OF *C. ANTHROPOPHAGA* IN SIERRA LEONE

Many observations of the clinical effects produced by the larva in man and animals in Sierra Leone have been made in recent years, and this Colony has for many years been considered to be a favourite haunt of *Cordylobia*. Smith (1908) remarks that 'Tumbu' is a Negro-Creole word, and gives a record of his findings of the larvae in Sierra Leone. He expresses doubt as to the correctness of the accepted mode of infection by the laying of the eggs or larvae in the skin; this doubt arose from his observations of the situation of the lesions in animals. Blenkinsop (1908) noted that in Europeans the upper part of the thigh and the buttock are the favourite site for the larvae to gain an entrance, and it is a generally received opinion that the parasites are often acquired at the latrine. The West Indian troops were often affected in the axilla, and natives, in any region. It was not known in 1908 whether *Cordylobia* was oviparous or viviparous, but Austen said that in either case, since the female is undoubtedly unable to pierce the skin with her ovipositor, the larva in its earliest stage must bore its own way through the integument by aid of its mouth hooks.

Smith made observations, as we shall see later, on the age incidence of the disease in man and animals, and also succeeded in breeding out flies from larvae obtained from rats and dogs. He mentions a wild rat which had six 'tumbus' in the bare underpart of its legs and feet, which were immensely swollen.

The prevalence of *Cordylobia* Myiasis in Sierra Leone is so considerable—the parasite itself is the cause of much discomfort to man, and causes suffering and even death in animals—that we took the opportunity of studying as carefully as possible the bionomics of the fly in its various stages, and of making experiments with a view to discovering the best method of attack upon it. We have also made observations upon the morphology of the first instar which may throw some light upon the mode of skin penetration of the larvae of other forms of Myiasis, notably those due to *Dermatobia*; owing to the fact that we found that the various stages did not always correspond to previous descriptions, we have included a short description of each of the stages of the fly. It is necessary for us to refer frequently to the work of Roubaud, as his

is the most recent and at the same time the most comprehensive work done on the subject. We have been enabled, largely owing to the greater amount of material at our disposal, not only to confirm his observations in many particulars, but also to add to them. If we are compelled to differ from him in a number of points, it is due entirely to the fortunate circumstance that a larger amount of material enabled us to carry our experiments further than he was able to do.

#### IV. MORPHOLOGY AND BIONOMICS

##### (1) ADULT (See Plate XV)

The following description of the adult stage is taken from Austen (1908): 'A thick-set, compactly-built fly of an average length of about  $9\frac{1}{2}$  mm.; specimens as small as  $6\frac{1}{2}$  mm. or as large as  $10\frac{1}{2}$  mm. in length are occasionally met with. Head, body and legs, straw yellow; dorsum of thorax and of abdomen with blackish markings; wings with a slight brownish tinge. The eyes meet together for a short distance in the median line above in the case of the male, but are separated by a broad front in the female. On the dorsum of the thorax the dark markings, which are a pair of longitudinal stripes not reaching the hind margin, are covered with a greyish bloom, and, consequently, not very conspicuous; this bloom is also present on the abdomen, but here the markings are much more distinct, especially in the female, in which the third segment, as also the fourth segment with the exception of the hind margin, is entirely black or blackish. In the female, the second segment is marked with a blackish quadrate median blotch, and has a similarly coloured hind border, broadening towards the sides, while the first segment has a narrow dark hind margin. In the male, these markings are not so extensive; the dark hind margin to the second segment is interrupted on each side of the median blotch, which is triangular in shape, and there is a yellow area of considerable size on the proximal half of the third segment, on either side of a blackish median quadrate blotch; the fourth segment is similarly but less conspicuously marked.'

## HABITS OF WILD FLIES.

The adult fly material which most observers have had at their disposal has, as a rule, been obtained by the process of breeding flies in the laboratory from larvae taken from the furuncular tumours of animals. Reference in the literature to the capture of adults is extraordinarily meagre. Rodhain and Bequaert (1913) observed wild adult females flying round the cages in which animals were kept, and followed the egg-laying process. Eggs were deposited in the straw and manure in the cages; experimental animals, monkeys and guinea-pigs, placed in the cages where the wild flies had laid, became infected, as the result of the larvae which emerged from the eggs penetrating the skin. In 1911 Roubaud, at Bamako, captured alive one fertilized female which laid eggs in captivity, and which supplied the egg and larval material for his experiments.

We have been exceptionally fortunate in this respect, because we have at Freetown, Sierra Leone, notorious in the history of Tumbu disease, been able to capture many adults indoors. Not only were numerous adult females and males captured, but several of the females were either fertilized before capture, or were fertilized after capture without difficulty. A point of interest is that these captures were effected and the experiments resulting from them were carried out in the dry season, during the months November, 1922, to April, 1923; in its proper place, further reference will be made to the bearing of this fact on the seasonal incidence of Myiasis due to *Cordylobia*.

On occasions the wild flies were seen on the wing; for example on the 27th March at sunset, on a cool evening several flies were seen in the open darting about after each other and buzzing loudly; they dashed into objects blindly, and one, a female, which had injured itself in this manner was captured.

Natives were able at times to capture adults in their houses, but the construction of their houses taken in conjunction with the resting habits of the fly as observed by us, explains the lack of success which often attended the efforts of the native to capture flies. The flies captured by us were found resting on the dark green painted ceiling of the bungalow verandah; on bright sunny days as many as three or four would be found there; on cloudy days they were rarely



found. They would remain there motionless for long periods, and only when disturbed would they fly about with great rapidity; they emitted during flight a loud buzzing noise, similar to that produced by the blow-fly; the noise ceased when they alighted again. Against the dark surface they presented a very inconspicuous appearance, and would commonly be overlooked. It was easy to understand that if this method of resting were followed on the smoky roofs of houses of native construction, the fly would be even less conspicuous. The flies were easily caught with a collecting net, and they gave the impression of being unable to see well in the day, as they allowed the net to approach close to them without taking flight. Wild flies were seen twice at night attracted by the light of a lamp on the verandah; they flew round noisily, knocking themselves against the lamp, and several had their wings scorched and fell inside the chimney. Whether these flies had come from out of doors to the light or had come to it from some resting place indoors is uncertain. It is probable the latter is the case, as after the systematic capture of all flies resting indoors in the daytime had been undertaken, no further captures were made at the lamp at night.

Wild or laboratory bred flies when placed in glass containers such as cylinders or inverted bell jars, of which the upper end was closed with cloth, rested chiefly on the cloth in the same upside-down attitude as did the wild flies on the ceiling. During the day they were rarely on the wing, but in the early morning from seven to nine, and in the late afternoon from four to six, they became very active, flying about, striking the glass sides of the vessel and buzzing audibly. At night they rested much as in the day, but the appearance of light near them at once aroused them to great activity.

#### FOOD OF ADULTS.

Roubaud observed a wild female fly feeding on sugar, on pulped fruit, and on ground soiled by urine. We found that both males and females, whether wild or bred, fed readily on banana and pineapple, the females feeding longer and oftener; both sexes also sucked up the juice from pieces of decomposing rat liver, and less readily fresh blood of a rat from a drop exposed on a slide.



## RESISTANCE OF ADULTS IN VARIOUS CONDITIONS.

*Direct sunlight.* Three wild flies were exposed in large test-tubes to the direct rays of the sun during the hours 11 a.m. to 2 p.m. They survived only from fifteen to thirty minutes. The results of this experiment serve to explain the fact that on bright days during the hot hours the flies come indoors to rest.

*Dry heat.* Wild flies were exposed to varying degrees of heat; they were placed singly in wide test-tubes which were plugged with wool and provided with a thermometer, the tubes were placed in a water bath which was rapidly brought up to the desired temperature. A male kept for thirty minutes at 44-45° C. was still active at the end of the period. On raising the temperature rapidly great restlessness was observed at 50° C., and at 52° C. the fly dropped suddenly dead to the bottom of the tube. The experiment was repeated with two other wild flies, one male and one female. The temperature was raised from 40° C. to 47° C. in two minutes; after one minute at 47° C. both fell dead suddenly. It appears from these experiments that a temperature of about 50° C. is fatal for the fly.

*Cold.* Four laboratory bred flies, one male and three females, were enclosed in a large tube containing a slice of banana and placed in an ice chest on 16th March, 1923. After an hour in the ice chest at a temperature of from 10° C. to 6° C. all were motionless. Two were removed to room temperature and rapidly recovered; they were then returned to the ice chest. The flies at each subsequent examination were motionless, sitting sometimes on the glass and sometimes on the banana; they were not observed to feed but changed their position slightly, and recovered their feet when shaken down. On 20th March, 1923, the one male died; the three females died on 23rd, 24th and 28th March, 1923, respectively. The powers of resistance to cold and damp are, therefore, very considerable.

**OVIPOSITION.** As we have seen, Rodhain and Bequaert observed oviposition on straw and manure in animal cages. Roubaud noted that his fly laid eggs on the glass walls of a vessel and on fruit. We found that of various sites on which gravid females in captivity were given the opportunity of depositing their eggs, the one most commonly selected was dry sand which had previously been

contaminated by the excreta of animals, in this case guinea-pigs. This fact was observed on an occasion when three females were placed in a bell-jar containing a guinea-pig in order to determine whether they would deposit eggs on the animal's skin. These flies had up to this moment been lodged in a container where they had access to cardboard, cotton wool, banana, and glass on which to oviposit; none of these sites was apparently suitable for them, as they did not utilize them. Immediately on their being admitted to the bell-jar containing the guinea-pig, two, and after a short delay, the third also, set about depositing their eggs in the contaminated sand with great eagerness and rapidity; eggs were not laid by them on banana leaves, carrot or orange, which were also present. It is perhaps not without bearing on this point that three other females in which ova apparently mature were present, died in the first container without laying their eggs. On three occasions, when sand was available and utilized, eggs were also laid on sites other than sand, but only in small numbers, viz., six on a piece of black cloth, seven on the white cloth cover of the bell-jar, and eleven on cotton wool. On only one occasion was a considerable number of eggs laid on any other material than sand, when contaminated sand was available; this was a case in which wet sand had been provided for the fly; she landed on it and protruded her ovipositor, but apparently found it too wet for her, as she immediately flew off; she laid one hundred eggs in a plug of pink cotton wool which was used as a stopper to the central aperture of the white cloth cover. It appears probable that the result of this experiment has some significance in regard to the wet seasonal incidence of this form of Myiasis in man and domestic animals. Apart from these occasions eggs were always laid in the sand provided for the guinea-pigs. In numerous experiments conducted during the laying of hundreds of eggs, flies could not be induced to leave the sand on which they were laying. The guinea-pigs did not attract them, nor did they oviposit on clean cloth nor on cloth impregnated with human perspiration, the pieces of cloth being placed in their path as they were laying their eggs. Flies on the other hand would not oviposit on sand contaminated with excreta, if the sand was too moist.

*Method of oviposition.* Generally for some hours, even a day,

before egg-laying commenced, the female could be seen pushing out and withdrawing the ovipositor, and from time to time small drops of clear fluid appeared at its tip. The procedure when ovipositing in contaminated sand was uniform for all the flies observed. The fly, having alighted on the surface of the sand, and having found a suitable area, digs with the tip of the abdomen a small cavity in the sand, backing slightly and curving the abdomen downwards to enable it to do so; the ovipositor is then extruded and pushed into the sand at the bottom of the small cavity. At this time, when the ovipositor sinks into the sand, the two hind legs bring up on either side a few grains of sand against the ovipositor, which is then withdrawn. The hind legs next move rapidly in a horizontal direction to scrape a little sand over the egg deposited in the small cavity, and to smooth the surface. The fly then advances hurriedly a few steps and commences again to dig in the sand, and repeats the whole process; she does not move in a straight line for long, but turns in her tracks frequently, with the result that a small area may be very thickly sown with eggs. The movements of the fly in the later stages of egg-laying often disturb eggs previously laid by it and uncovers them, bringing them to the surface. On cotton wool the eggs were laid on strands about one quarter-inch from the surface.

*Batches and number of eggs laid.* From the fact that his fly, which laid over one hundred and fifty eggs, died after ovipositing, Roubaud concluded that *Cordylobia* cannot survive parturition; also he concluded that probably, as the number of eggs laid was much higher than what he found in the case of *Auchmeromyia*, only one batch of eggs is laid by *Cordylobia*. Our observations show that at least two batches of eggs may be laid, and that the female does not die immediately after parturition. For example, a wild fly, No. 22, was observed to lay two batches of eggs in captivity. It was captured on 29th January, 1923, oviposited on 1st February, 1923, and again on 11th February, 1923; it died on 16th February, 1923. A laboratory bred fly, No. 38, emerged from the pupa on 23rd February, 1923, and was fertilized while still unfed on the same day by a wild male; she laid the first batch of eggs on 5th March, 1923, and a second on 8th March, 1923; she died on 10th March, 1923. Another laboratory bred fly which emerged on

10th March, 1923, and was fertilized on 11th March, 1923, laid a first batch of eggs on 17th March, 1923, and a second on 20th March, 1923; she died on 24th March, 1923. The number of eggs laid in the first batch varied from two hundred and eighty-seven to three hundred, in the second batch from ninety-four to one hundred and eighty-four. It appears probable that the batch of eggs laid by Roubaud's fly was the second batch.

*Dissection of gravid females.* Several females which died without laying eggs were examined. One laboratory bred female which emerged on 20th February, 1923 and copulated on the same date died on 3rd March, 1923, without laying; she contained three hundred and four eggs; another laboratory bred female which emerged on 10th March, 1923, and was not seen to copulate, died on 24th March, 1923, containing five hundred and three eggs in different stages of development; a wild fly, with which a wild male would not copulate, died on 23rd February, 1923; she contained four hundred and four eggs.

*Rate of Oviposition.* On several occasions when females were engaged in laying in the sand, the total time taken in laying a batch of eggs was noted, and also the rate per minute. The total time taken by one fly in laying two hundred and eighty-seven eggs was thirty-three minutes; by another for one hundred and eighty-four eggs was twenty-six minutes. During the time there were several pauses of varying length, and this reduced the average number of eggs laid per minute. On the whole, however, the rate was very constant for all the flies observed. Thus, taking a total of forty-six individual minutes timed among several flies at the time they were ovipositing, the smallest number of eggs laid in a minute was five, the highest eleven, the average per minute being eight. This relatively slow process is against the idea of egg-laying on animals.

#### LENGTH OF LIFE OF FLY IN CAPTIVITY.

The longest period during which a wild fly lived in captivity was eighteen days; this was a female, which during that period laid two batches of eggs, surviving the last oviposition for five days. Several laboratory bred females lived fourteen days, but only one lived for fifteen days.



## (2) EGG

This is white in colour and measures on an average 0.8 mm. in length; it is banana shaped, being almost straight on one side and curved on the other; it tapers somewhat towards one end. On the surface there are longitudinal grooves, and there is also a fine hexagonal reticulation. In eggs from which the larva has emerged it is seen that there is near the smaller pole a longitudinal slit extending about one-third along the flattened surface; through this slit the larva has emerged.

**SITE.** The eggs were found just under the surface of the sand in which they were laid; in cotton wool also, not on the surface but about a quarter of an inch deep. The eggs adhered in most cases to particles of sand, or in cotton wool to the strands, and could not be shaken off.

**HATCHING.** For some hours before hatching the egg shows a darker patch towards the more pointed end; as the time of hatching approaches it is seen that this dark patch is in active movement, and it is recognised as the chitinous buccal armature of the larva tearing at the inner surface of the eggshell. By means of this armature the larva cuts a linear opening on the flat surface of the egg near the small pole; as soon as it is possible to do so, it pushes its cephalic end through the aperture, which it proceeds to enlarge by vigorous movements of the anterior body segments. In cases watched throughout the process, it usually took from four to six minutes from the moment when the aperture was first observed till the time when the larva had cleared itself of the eggshell.

**RESISTANCE OF THE EGG TO VARIOUS AGENTS.**

*Room temperature.* On glass the larva emerged in three days as a rule. Roubaud found a shorter period on sand, and noted that eggs on wet sand hatched somewhat later than eggs on dry sand.

*Incubator at 37° C.* If eggs were placed in watch-glasses either dry or immersed in a small quantity of water they hatched in twenty-four to forty-eight hours, the water drying up.

*Sunlight.* Exposure to the rays of the sun for one hour, whether on glass or on dry or wet sand, did not prevent them hatching within four days. Two larvae in this experiment were watched



leaving the egg; the process was short, less than a minute elapsed before the larvae were delivered from the egg, but they dragged the eggshell about for another half minute, before getting rid of it. Eggs exposed to the sun for four days did not hatch, even when subsequently removed to the shade at room temperature.

*Dry heat.* Numerous experiments were carried out with eggs in plugged tubes placed in a water bath. Exposure to temperatures of 60° C., 55° C., 50° C., and 45° C., for two minutes killed all eggs used.

*Wet heat.* Similar results were obtained by heating eggs submerged in water in tubes placed in a water bath at these temperatures for two minutes.

*Hot ironing.* Eggs were rendered incapable of hatching by passing over them lightly a flat iron at a temperature suitable for pressing clothes. The eggs were not protected by being in cotton wool, nor even by three folds of cotton cloth; they were flattened and desiccated in the process, a point of some practical importance in view of the frequently accepted theory that clothes are infected when at the laundry.

*Cold.* Eggs placed in an ice chest did not hatch in seven days, nor after removal to room temperature. Four eggs were placed in the ice chest for forty-eight hours; on removal to room temperature two emerged in three days.

Eggs dissected out of dead females did not develop in any medium, either at room temperature or at 37° C. in the incubator.

### (3) LARVA

**FIRST INSTAR.** Many descriptions of larvae from cases of Myiasis have been made from larvae which were in the later stages of development. It is, however, very important that not only the later instars should be examined, but that the first instar should receive attention. At this stage, in those larvae which can produce true cutaneous Myiasis, very interesting adaptive structures are found, some of which appear to render the larvae capable of penetrating unbroken skin, while others determine the ability of the larvae either to remain in situ in the skin or to penetrate further into the tissues. The structures which attract attention chiefly are the cephalo-pharyngeal skeleton and the cuticular spines. The first

stage larva of *Cordylobia* presents points of interest in respect to both these structures, as will be seen in the description given below. Phenol was used as a clearing agent.

The newly hatched larva is white in colour and is visible to the unaided eye. It measures from 0.75 mm. to 1 mm. in length; it is somewhat fusiform, tapering from the mid region towards the anterior, and to a less degree towards the posterior extremity; it is composed of thirteen segments. The first or cephalic segment is the smallest; the mouth aperture is situated near the ventral surface of this segment; the ventral surface of the segment adjacent to the mouth is yellowish, chitinized, and densely clothed with yellow spines directed backwards. On the dorsal region of the segment there are anteriorly two rounded projections, one on either side. On the posterior portion of each projection there is a minute antenna-like structure consisting of two segments: on the anterior portion is a small chitinized pit. Near the caudal margin of the segment are several rows of backwardly directed yellow spines. Segments number two to eight are covered with backwardly directed spines, almost colourless except towards the cephalic margin of each segment where the spines are distinct and more heavily chitinized, yellow or even brown in colour. Segment nine is almost devoid of spines, being provided at the cephalic margin with a single row of backwardly directed spines and at the caudal margin with a single row of spines, in this case *forwardly* directed. Segments ten and eleven have no spines at the cephalic margin, but on the caudal margin have several rows of spines directed *forwards*, the rows being more numerous on the dorsal aspect.

Segment number twelve is longer on the dorsal than on the ventral aspect; it is densely clothed all over with large, strongly chitinized and *forwardly* directed spines. These large spines directed forwards and strongly chitinized appear to act in keeping the larva in position with its posterior end at the surface of the skin. It is interesting to note that, judging from the drawings in Surcouf's (1913) article of the first instar larva of *Dermatobia cyaniventris*, a similar arrangement exists there. This segment in *Cordylobia* is furnished with several soft digital processes; of these two are visible on the dorsal surface, one on either side of the middle line, two are situated laterally on the segment,

one on each side, while two are situated on the ventral surface, one on each side of the anal orifice.

Segment number thirteen is small and has only a few sparsely distributed spines; on this segment there are four pairs of soft digital

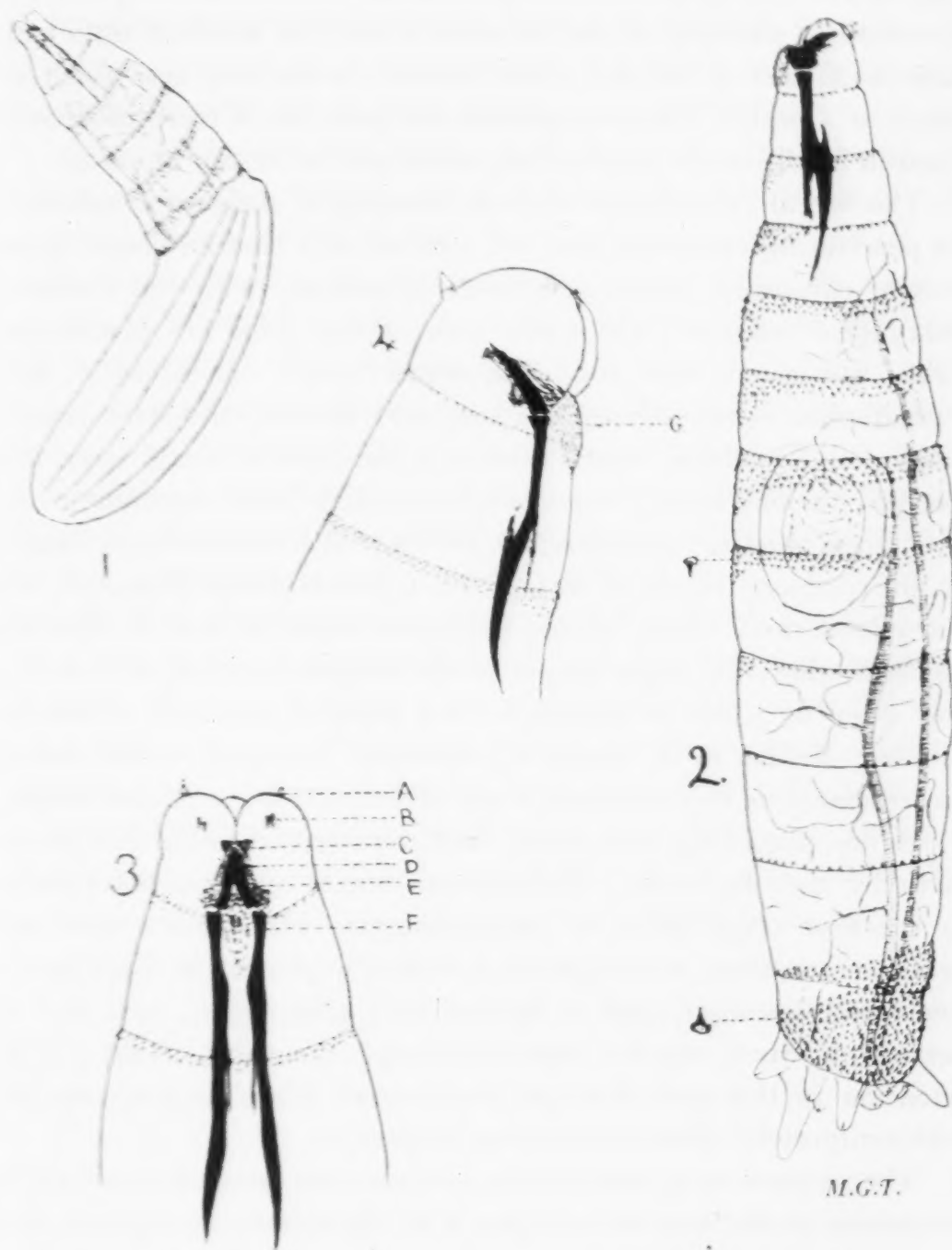


FIG. 1. (1) Egg hatching. (2) First instar larva. (3) Head of larva of first instar, ventral view. (4) Head of first instar larva, lateral view.

*A*—antenna, later papilla; *B*—chitinous pits; *C*—prestomal sclerite; *D*—median buccal spine; *E*—oral rods; *F*—cephalo-pharyngeal sclerite; *G*—ventral spiny area adjacent to mouth.

processes in addition to the tracheal tubes; the tracheal tubes open dorsally on the segment near its anterior margin on flattened eminences. The digital processes have in many cases at their tips a chitinous pit; the processes are of assistance to the larva in locomotion, and it is by means of them that the larva can attach its posterior extremity to particles of soil or other objects on which it rests and holds itself erect in the air, while waving its anterior end about in search of a host. The arrangement and position of these processes, fourteen in all, on the last two segments can be seen in figure 4.

The buccal armature or cephalo-pharyngeal skeleton is adapted for penetrating unbroken skin. It consists of a median buccal spine heavily chitinized, which articulates posteriorly with the cephalo-pharyngeal sclerites. On either side of the spine are placed the feebly chitinized oral rods (baquettes orales of Keilin). The cephalo-pharyngeal sclerites are long and slender, and have poorly developed dorsal and ventral cornua. The median buccal spine has received various names, for example, median hook, median tooth, and labral sclerite; according to Keilin, 'the median hook occurs in the primary larva of nearly all Cyclorrhous Diptera.' In *Cordylobia* first instar larvae, the spine when at rest is directed upwards almost at right angles to the cephalo-pharyngeal sclerite, and when in action its movements are directed upwards to lift the cuticle. Neither in its shape, nor direction, nor in its action, has it any resemblance to the mouth hooks of the second and third instars, which are, according to Lowne (1890), represented in the first instar larva by the oral rods. This median buccal spine is not present in the first stage larva of *Auchmeromyia*, which larva does not penetrate the skin; it is significant that it is present in *Hypoderma bovis* and *lineatum*, and is figured by Laake (1921), and that it persists in them up to, and including, the third instar. The retention in this case is easily understood when we consider the active migratory character of these stages.

The respiratory system consists of two main tracheal tubes which commence at the posterior stigmata on the thirteenth segment and run forward as parallel bilateral tubes; up to the tenth segment they are yellow in colour; in the region of the eleventh segment there is a transverse tube connecting them, which is also yellow in colour. Along their entire course forward they give off branches, and thus



they become attenuated anteriorly; no anterior stigmatic opening can be made out.

*Habits of the first instar larva.* The larvae remain in the situation in which they hatch out just below the surface of the sand; they are difficult to see, and even close inspection of the surface with a hand-lens may fail to reveal their presence. If, however, the container in which they are present is vibrated by tapping it, or by shaking the table on which it stands, or if the surface of the sand is disturbed by blowing on it or touching it with a needle, the larvae make their way up rapidly through the few grains of sand covering them. Similarly they come quickly to the surface and wave about, if a vessel containing hot water is brought near the surface of the sand in which they lie concealed. When the larvae are so disturbed it is easy to observe them with the naked eye; they adopt a characteristic attitude, being attached by the posterior end to a grain of sand, the rest of the body being raised in the air, and waving about actively as if seeking for something to which to attach themselves. If any object is allowed to touch the larvae they at once adhere to it and quickly crawl up on it. Camel's-hair brushes were used at first for picking up the larvae, but the larvae have the habit of at once creeping in between the hairs and disappearing from view in a few seconds; brushes were, therefore, discarded in favour of handled needles for the purpose. There is need for great care, however, that they are not injured in transferring them from the needle on to any other object such as a skin surface, as the slightest accidental pressure may render them very slow in penetrating skin or even incapable of doing so.

*Resistance of larvae of the first instar to various agents.*

**Room temperature.** Left in sand at room temperature, larvae lived without food for about nine days, as a rule; some died much earlier, and a few lived as many as fifteen days.

**On cloth.** Larvae two days hatched were taken up on cloth by laying it gently on the surface of sand containing larvae; the cloth with larvae adherent to it was kept at room temperature; the larvae lived on the cloth for nine days; a portion of wet cloth was used to pick up larvae, and allowed to dry with the larvae on it; the original condition of the cloth in this respect made no perceptible difference in the length of time larvae could live on it.



**Direct sunlight.** Larvae on a watch-glass exposed for twenty hours to open air where the sun reached them during the whole day did not die, and were capable of penetrating skin. Larvae in sand, exposed to the sun for two hours in the heat of the day on one occasion only, and thereafter kept at room temperature, lived for over eleven days.

**Dry heat.** Larvae were placed in small dry tubes plugged with cotton wool; the small tube was placed in a test-tube large enough to contain also a thermometer, and the large tube was placed in a water bath. A range of temperatures and exposures was tried, and it was found that rapid definite effects were obtained at a temperature of  $50^{\circ}\text{C}$ . and above. After two minutes' exposure at  $50^{\circ}\text{C}$ . the larvae became motionless and failed to recover.

**Incubator at  $37^{\circ}\text{C}$ .** On dry sand in a watch-glass the larvae lived for three days only.

**Hot water.** In this experiment the small tubes containing the larvae to be tested were filled with water and plugged, and placed in a large test-tube half full of water, which was placed in the water bath. For this series forty-three larvae were used at various temperatures. They survived one minute at temperatures below  $48^{\circ}\text{C}$ ., but one minute at  $50^{\circ}\text{C}$ . and higher temperatures killed them. Temperatures from  $45^{\circ}\text{C}$ . to  $48^{\circ}\text{C}$ . for two minutes gave irregular results.

**Cold.** Larvae placed in a watch-glass and laid on ice, and kept there for twenty hours, became motionless but did not die; on removal to room temperature they quickly became active and were able to penetrate the skin of a guinea-pig.

**Immersion in cold tap water.** Larvae attached to needles were sunk in tubes of water, and frequently remained attached to the needle for long periods. Consecutive immersion for ten and thirty minutes produced no result; larvae immersed for ten minutes and allowed to dry, and then put in water for thirty minutes, were active at the end of the time; one made an unsuccessful effort to penetrate skin. For longer periods this method was inadequate as the larvae floated up to the surface of the water, where some of them remained active for three days. Complete immersion in tubes for long periods—in one instance up to twenty-four hours—was not fatal to them.

**Ironing.** The process of ironing was fatal to larvae in cloth, even when covered with several layers.

**Phenol.** Solutions of phenol of a strength greater than 12 per cent. killed larvae so quickly that they were unable to crawl out of the solution: solutions of less strength did not prevent them crawling out. Watch-glasses were discarded in the subsequent experiments, which were carried out as follows:—A drop of the reagent to be tested was placed on a slide, the larva placed in it, and over the larva a small square of filter paper soaked in the reagent was placed; the filter paper diminished the activity of the larva in crawling out of the fluid. Ten per cent. phenol and 5 per cent. phenol killed larvae in five minutes, while 1 per cent. sometimes failed to kill in ten minutes and frequently failed to do so in five minutes.

**Sodium hydroxide.** Solutions from 20 per cent. down were tried; all strengths down to and including 1 per cent. killed in five minutes.

**Formalin.** Five per cent. solution killed in all experiments in ten minutes, but not always in five minutes; 1 per cent. did not kill in ten minutes, but did in twenty minutes.

**Chloroform water** in the strength of 40 per cent. chloroform killed in ten minutes, but not always in five minutes.

**Calomel powder.** Larvae placed on calomel powder did not die, but moved about actively in it for an observation period of forty-eight hours.

The effect on the first instar larva of oily substance is mentioned under prophylactic experiments, and the resistance of second and third instar larva is more properly dealt with under Treatment, as these stages are already lodged in the tissues of the host.

*The skin penetrating power of first instar larvae.* Experiments made with larvae which had hatched from a few hours to as many as fifteen days previously, showed that they were capable of penetrating the healthy skin of various living animals as long as the larvae remained active. Of the fifteen days old larvae tried, only one penetrated, and that not completely; at twelve days old many larvae penetrated easily and completely. Numerous experiments were carried out on the skin of man, European and native, and on chimpanzee, dog, cercopithecus spp., cat, bush cat, guinea-

pig, wild rat and fowl; in all these cases penetration of the unbroken skin was accomplished. Larvae proved unwilling or unable to penetrate the skin of frog, lizard and python. Where penetration of the skin was successful, great variation was noticeable in the time required for the larva to conceal itself under the skin. The animals in which entry to the skin was effected most expeditiously were very young wild rats, brown or black, and in these, as in other animals, the different regions of the body offered differing degrees of resistance to the boring powers of the larvae. For example, six larvae penetrated the shaved skin of the rat abdomen in from twenty-five seconds to one minute; other six placed on the soles of the feet of the rat required from thirty seconds to two minutes. Again on the shaved skin of the thorax or abdomen of the guinea-pig, larvae penetrated in from thirty seconds to a minute and a half; larvae placed on the soles of the feet required from seven minutes to twenty-five minutes. Occasionally larvae succeeded only in partially penetrating the skin; but in no case observed, where the larva succeeded in concealing itself, did the process occupy more than half an hour.

**Method of penetration.** When an uninjured larva is deposited on the skin of an animal which is suitable, the larva quickly crawls into the nearest groove or wrinkle in the skin, puts down its head and commences to bore in. The median black mouth spine can be seen in active movement, the cephalo-pharyngeal sclerites moving in unison with it; the movement of the spine is directed to piercing the cuticle and enlarging the aperture on both sides; there is a considerable range of lateral movement of the spine, and a corresponding movement of the sclerites to which it is articulated. Once the entrance aperture is large enough to admit the cephalic end of the larva, the body very rapidly insinuates itself under a thin tunnel of cuticle; the rapidity depending on the thinness and softness of the skin. The action of the mouth-parts and the method of using them were studied very carefully in many experiments; particular attention was given to this, as the result of the observation that in the dead fixed larva of the first instar the median buccal spine was usually directed dorsally, and was not curved down ventrally as are the mouth hooks of the second and third instars. The experiments were carried out by snipping off a

very thin layer of skin from recently killed rats and placing it on a slide under the microscope and then placing on it a larva at a point where the action of the mouth-parts could be followed; if the larva entered at a point not desired, the skin could be manipulated with

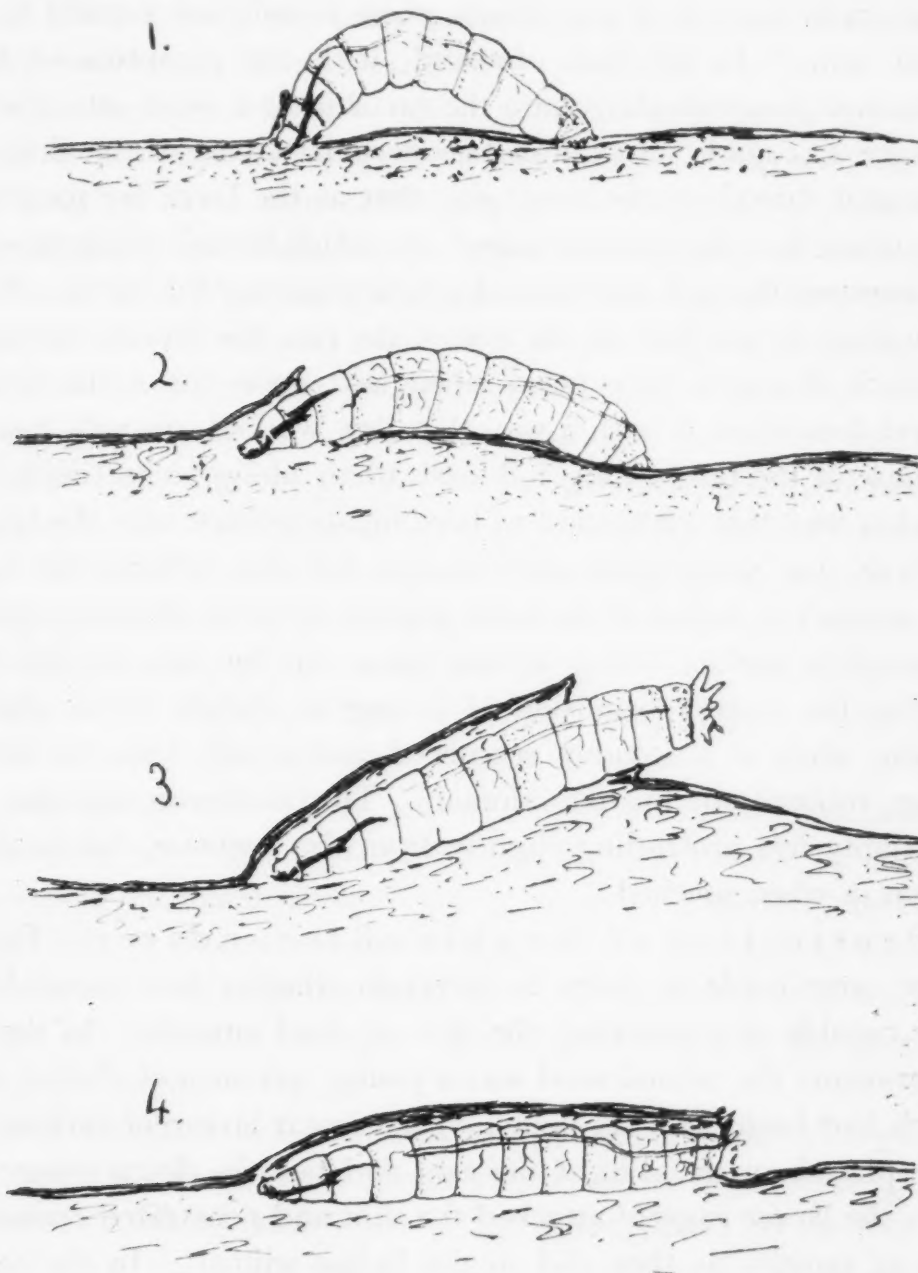


FIG. 2. First instar larva penetrating skin.

M.G.T.

needles, so as to bring the point of entry into view. In this way it was seen that the median buccal spine was used for penetrating the cuticle and then separating it from the subjacent tissues, by a series of punching movements directed forwards and extending



laterally until an entrance aperture was made; then followed a succession of upward movements of the mouth spine. An illustration which may serve to explain the upward movements is that they resemble the upward movements of the points of a tack-lifter, but in this case, instead of two points, there is only one formed by the buccal spine. In all cases observed, with one exception—a larva penetrated perpendicularly into the ear skin of a dead rat—the first entrance was made into the most superficial layers of the skin in a horizontal direction: the result was that as the larva lay parallel to the surface in a thin-roofed tunnel, the black buccal armature could be discerned through the layer of cuticle covering the larva. It was interesting to see that on the tail of the rat, the larvae, no matter in which direction they faced when laid upon the hairs, quickly turned themselves in such a way that they worked upwards towards the base of the tail. They followed a hair along to its origin from the skin and then proceeded to bore in, sometimes near the base of the hair, but more commonly through the skin between the hairs. The instinctive action of the larva appears to be to obtain as quickly as possible shelter and a resting place in the skin superficially, leaving the further operation of boring in deeply to be done at leisure, when it is securely ensconced and is safe from the risk of being rubbed off by the animal. The posterior segment was invariably left protruding slightly from the aperture, but could be drawn in when touched.

**Penetration of the skin of the cadaver.** Experiments were made in order to ascertain whether first instar larvae were capable of penetrating the skin of dead animals. In the first experiments the animal used was a young specimen of *Rattus rattus* which had been dead six hours. First instar larvae of various ages were placed on the skin of the ears and tail; in eleven consecutive trials the larvae eagerly attacked the skin and penetrated completely and as rapidly as they did in the living animal. In the second experiments the foot of a dead guinea-pig was used; here again penetration was accomplished with normal rapidity.

**Existence in cadaver.** Although in no case did larvae which had so penetrated complete their development, they proved capable of living in the tissues of a dead animal for two or three days; on one occasion a larva which had penetrated a cadaver reached the stage



of the first ecdysis. If an animal which had larvae of the second or third instar present on it died, the larvae as a rule at once migrated from the dead body and buried themselves in sand or soil; in the case of one guinea-pig, however, several second and also third stage larvae did not leave the host, but remained active in the tissues for twenty-four hours, before they died.

*Secondary penetration of skin.* Rodhain and Bequaert removed larvae at an advanced stage from their site in the skin of the host and inserted them into artificially made cutaneous pockets in a different host, and observed that they were capable of developing. Roubaud, as a result of his experiments, concluded that not only older larvae once removed from the host are incapable of penetrating skin afresh, but also that young larvae of the first instar are equally incapable of doing so. This he attributes to a sudden biological modification of the larva. We are in agreement with this observer as regards the lack of *penetrating* power of the second and third instar larvae, but our observations differ from his in regard to the first instar. Second and third instar larvae expressed from cavities in living guinea-pig skin made strong and often successful efforts to regain the position where they had been lodged; they made similar efforts to regain their position in tissues of dead animals on some occasions. They proved, however, quite incapable of penetrating unbroken skin, either of the same animal or of other animals. First instar larvae, however, proved capable of penetrating skin afresh, if removed from the original site within some hours of their entrance; early in the instar they are capable of re-penetration of skin, but later they are not. In Table I are given the details of some experiments.

From the table it is seen that in three experiments with guinea-pigs and rats, first instar larvae succeeded in re-penetrating skin completely; second and third instar larvae failed to do so. We consider that the factor which renders it possible for larvae early in the first instar to re-penetrate, whereas late in the first instar they are incapable of doing so, is the possession in a functional state of the median buccal spine. This apparatus is as well adapted for the purpose of skin penetration, as the mouth hooks of the second and third instars are ill adapted for the purpose.

*The effect of reagents in regard to skin penetration.* The

cadaver of the young rat was used and powders and oily substances were applied to the skin, and the larvae placed on skin so treated. It was found that French chalk, borax or calomel, delayed them, but did not prevent them penetrating the skin. Oily substances, however, had a great effect, the effect being similar for palm oil, vaseline and liquid paraffin. The larvae placed on skin treated with these did not proceed to bore into the skin; they commenced wandering about, trying to get out of the layer of liquid, and could do nothing as long as they remained in it. The disadvantages, however, are obvious, as the film of oily substance must be of considerable thickness; it is improbable that on such lines a practical prophylaxis can be evolved, as when larvae are free from the liquid they can penetrate the skin, although more slowly.

TABLE I

Giving the results of experiments to test the secondary skin-penetrating powers of the larvae of *C. anthropophaga*, Grünberg.

No. of Exp.	No. of larvae	Instar of larvae	Animal from which removed	Animal on which tested.	Time required for penetration		Remarks
					Min.	Max.	
1	2	3rd	Brown rat	Black rat	...	...	Did not penetrate
2	2	3rd	Brown rat	Guinea-pig	...	...	Did not penetrate
3	2	2nd	Guinea-pig	Guinea-pig	...	...	Did not penetrate
4	1	2nd	Guinea-pig	Black rat	...	...	Did not penetrate
5	6	2nd	Guinea-pig	Black rat	...	...	Did not penetrate
6	1	1st	Guinea-pig	Guinea-pig	...	...	Did not penetrate (end of instar)
7	4	1st	Guinea-pig	Guinea-pig	...	...	Did not penetrate (end of instar)
8	2	1st	Guinea-pig	Guinea-pig	1 min.	3 min.	Penetrated
9	2	1st	Black rat	Black rat	8 "	...	Only one completely penetrated
10	2	1st	Black rat	Black rat	2 "	3 min.	Penetrated

*Food of first instar larvae.* First instar larvae grow slightly larger if left in contaminated sand at room temperature, but it is not possible to say whether this increase of size is due to the ingestion of food material which may be taken up in small quantities from the sand. The larvae did not show any capacity for existing

long, or developing on such substances as fruit of various kinds, pieces of muscle, blood or liver of rats. Living tissue affords their natural food.

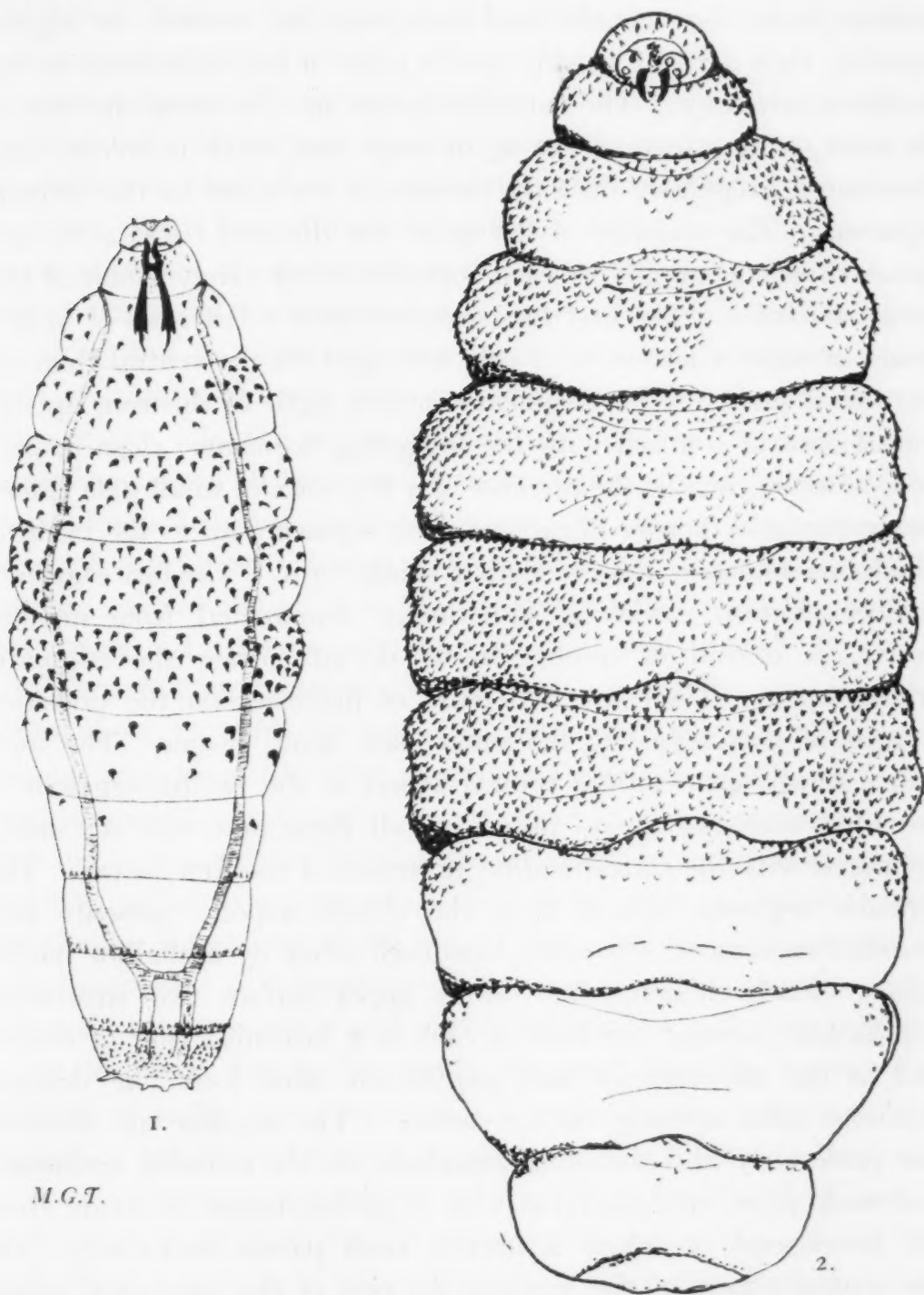


FIG. 3. (1) Larva of second instar, ventral view. (2) Larva of third instar, ventral view.

**THE SECOND INSTAR LARVAE.** This stage arises by a moult which occurs in the tissues of the host generally about the third day after penetration; the larva then measures 2.5 to 4 mm.; the

appearance and shape of the larva differ entirely from that of the first instar; whereas the first instar larva is somewhat fusiform in shape, the larva of the second instar is club-shaped; it expands quickly from the cephalic end to about the seventh or eighth segment, then narrows quickly and is more or less cylindrical to the posterior extremity. The cuticular spines in this instar are one of the most striking features, being of large size, black in colour, and distributed irregularly over the surface of the third to the seventh segments. The majority of the spines are directed backwards, but spines directed laterally or even forwards occur rarely; some of the spines are bifid. The first two segments bear a few rows of brown spines of smaller size more closely arranged on the ventral than on the dorsal surface. The segments number eight to thirteen, appear almost bare in contrast with the foregoing segments; close inspection, however, reveals the presence of a few rows of small pale spines on the posterior border of each of these segments up to the twelfth, which has numerous rows of similar spines on it. The last segment, number thirteen, which is indistinctly demarcated from number twelve, is devoid of spines; on its dorsal surface the stigmatic orifices open. There are two pairs of processes on the posterior margin of this segment, the outer pair being larger. The anal orifice which opens on the ventral aspect of the twelfth segment is provided with two lateral processes; all these processes are small, compared with the corresponding processes of the first instar. The cephalic segment viewed from the dorsal aspect, presents two rounded eminences, separated from each other by a shallow mesial sulcus. Each eminence has on its upper surface two structures, papilla-like; around the base of each is a brownish ring of chitin, and in the substance of each papilla are what look like delicate chitinous tubes opening on the surface. The papillae are situated one posteriorly and the other anteriorly on the rounded eminence, and each arises in connection with a goblet-shaped structure from the lower end of which a narrow cord passes backwards. On the ventral aspect of the segment the tips of the two black buccal hooks protrude; external to them on either side is a yellow ridge bearing small spines.

The mouth parts consist of two black hooks strongly curved ventrally, in contrast with the median spine of the first instar.



Posteriorly the mouth hooks articulate loosely with a hypostomal sclerite consisting of short rods united by a transverse bar. This sclerite articulates in turn posteriorly with the pharyngeal sclerites which pass backwards, reaching the middle of the third segment in extended specimens.

The respiratory system consists of two main longitudinal tracheal tubes which pass forwards from the posterior stigmata on the thirteenth segment to the posterior lateral border of the second segment, where they end in the anterior stigmata. The posterior stigmata consist of two curved slits, slightly oblique in direction, the concavities of the curves facing each other. The main tracheal tubes are chitinized to about the middle of the eleventh segment; just anterior to the chitinized portion a transverse tube connects the two lateral tubes. In the region of the third segment the main tubes, which have given off branches all the way forward, suddenly narrow and continue as a fine chitinized tube to the anterior stigmata, each of which consists of a fringe of finger-like processes about eight in number. Great variation in size is seen in late second instar and early third instar larvae even from the same host.

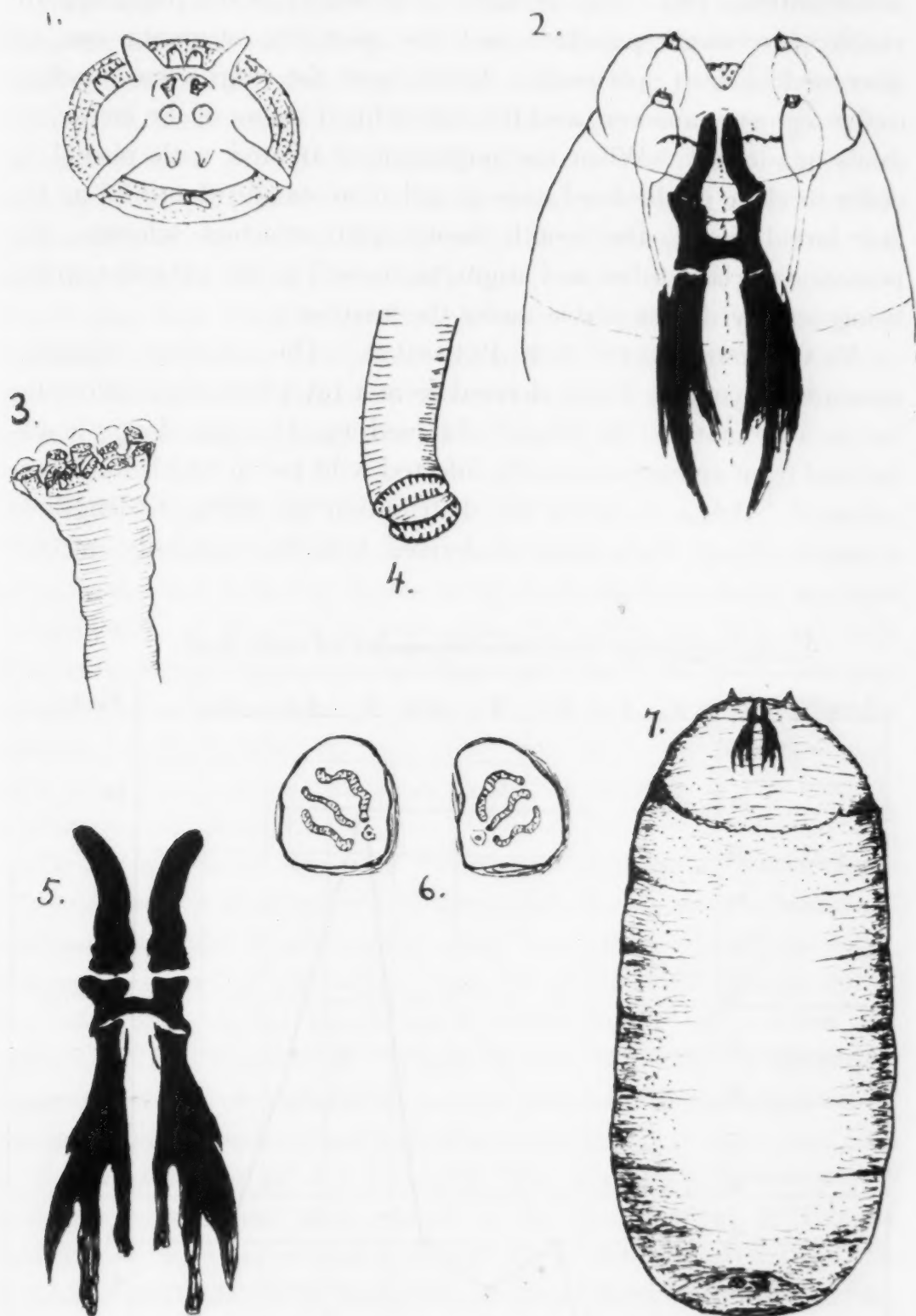
**THIRD INSTAR LARVAE.** The second ecdysis occurs in the host tissues from the fifth to the sixth days, and the resulting larva matures and leaves the host about the eighth day. The extended larva measured, when mature in rats, from 13 to 15 mm. long. Not only the measurements, but also the date on which the larva reaches maturity and the date on which the ecdysis occurs is influenced by the relative suitability of the host. The mature larva consists of twelve segments; it is roughly cylindrical in shape, its greatest width occurring at the seventh and eighth segments. The first segment is the smallest, and is frequently retracted into the second. It presents dorsally on each side a protuberance carrying two segmented papillae, the basal segment being chitinized. Ventrally on the segment are seen the free extremities of the two black buccal hooks sharply pointed and curved ventrally; on either side of the hooks there is a ridge of yellow chitinized integument bearing a row of small spines, about six in number. The mouth hooks articulate posteriorly with the hypostomal sclerite, which in turn articulates posteriorly with the pharyngeal sclerites; the posterior end of these reaches to the junction between the second and third segments. On



the second segment at its posterior margin laterally, the anterior stigmata open; these consist of a fringe of finger-like processes about ten in number. The twelfth segment is small; near the anterior margin of its dorsal surface the posterior stigmata open close together on rounded eminences; they consist of three sinuous slits obliquely placed; internal to the slits is a small circular opening; there does not exist in this case a definite chitinized plate on which the stigmatic slits open. The cuticle of segments number four to eleven, on the ventral surface, is thrown into folds of an irregular appearance, which are more pronounced in the middle segments, and are concerned in locomotion. Backwardly directed curved spines are present on segments two to nine, being more numerous and of darker colour on the sixth, seventh and eighth segments; on the ninth segment few spines are present, on the tenth still fewer, while the eleventh and twelfth are practically bare. The white appearance of the last four segments forms a marked contrast with the speckled black appearance of the anterior segments. The great diversity of appearance which the larva of *Cordylobia* presents in its different instars and at different stages of the same instar, has induced some observers to introduce separate names for them; this is of little assistance, and tends to add to the already sufficiently great confusion which exists in regard to the larvae of flies causing Myiasis.

#### (4) PUPARIUM

PUPATION. The process of pupation was observed in larvae which had been removed either from naturally infected or from experimentally infected animals. If mature the larvae commence to pupate within twenty-four hours; the anterior extremity becoming pinkish at first, then terra-cotta coloured; the colour extends along the body to the posterior end, and later darkens to a dark chestnut. If the larvae are immature, pupation may be delayed for a day or two, or the larvae may die and turn dark in colour; if the larva has been chloroformed or has been removed from a sloughing septic cavity, it becomes rapidly black from before backwards, the puparium fails to separate and harden, and the larva dies. The shape of the puparium is rather characteristic. It has the posterior end very squarely cut off and the sides run parallel to each other, giving an elongate oblong appearance; it tapers somewhat abruptly



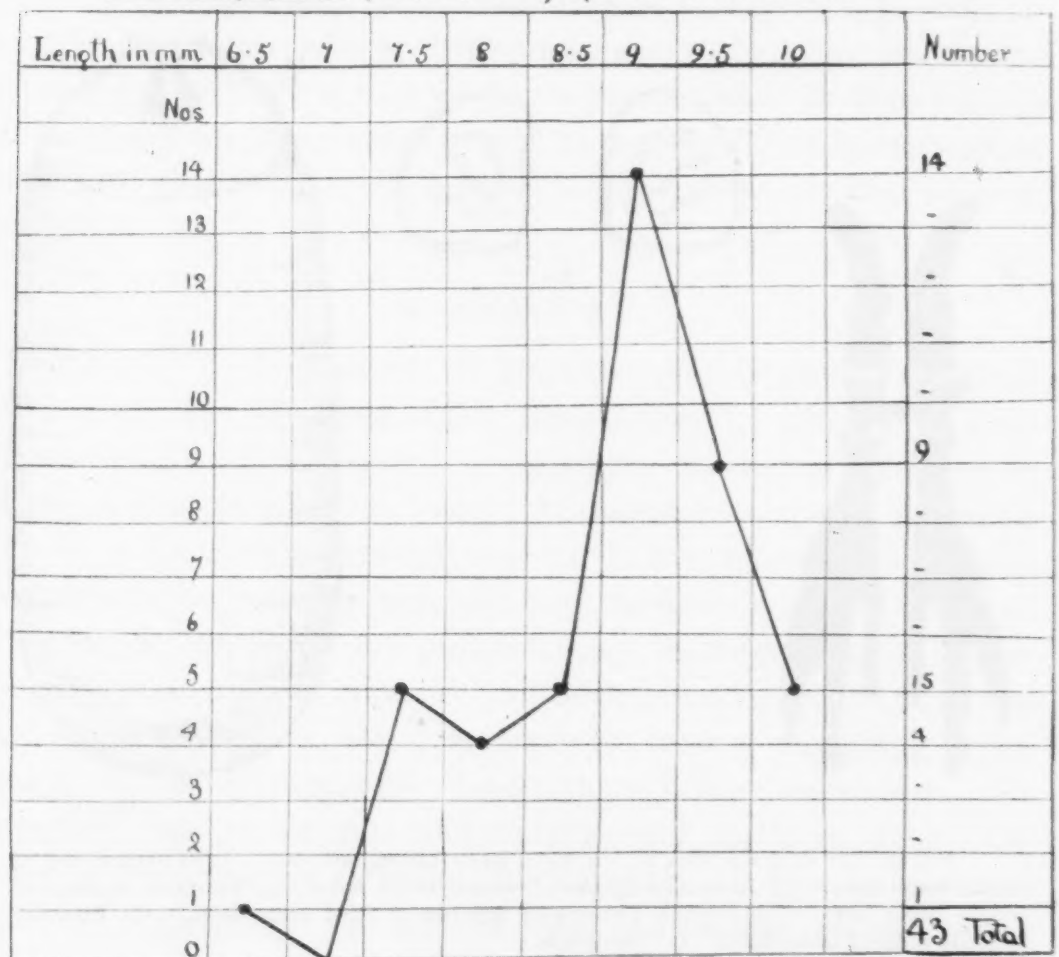
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FIG. 4. (1) Posterior view of first instar larva showing processes. (2) Cephalic end of second instar larva. (3) Anterior stigmata of second instar larva. (4) Posterior stigmata of second instar larva. (5) Cephalo-pharyngeal skeleton of third instar larva. (6) Posterior stigmata of third instar larva. (7) Puparium.

at the anterior end. On the anterior extremity of the puparium are visible two small papillae, and the posterior stigmata can be discerned in most specimens. In all cases the rings corresponding to the segments are seen, and the well defined spines of the larvae are easily made out. When the puparium is treated with phenol in order to clear it, the hard case is still more easily identified as the last larval moult, the mouth hooks and attached sclerites, the posterior tracheal tubes and stigmata, as well as the cuticular spines being readily demonstrable under the microscope.

MEASUREMENTS OF THE PUPARIUM. The smallest obtained measured 6.5 mm.; it was derived from a rat which died before the larvae were mature; the largest obtained was 11.5 mm. long; it was derived from an experimentally infected wild rat in which the larvae matured. Below is shown the distribution according to length of a series of forty-three puparia derived from the naturally infected wild rat mentioned above.

*C. anthropophagi* from a naturally infected rat which died.



GRAPH I. Showing the distribution according to length of 43 puparia of *Cordylobia anthropophaga*, Grünberg.

**SITE CHOSEN FOR PUPATION.** Larvae which had been removed from the host, or had left the host, were observed; when placed on sand which was dry they began after a short time to dig down into it, and in most cases in an hour or two they were out of sight. Sand was then provided in small tubes for each larva; the sand was so arranged that there was a damp layer of sand at the bottom of the tube of one inch in depth, and a dry layer of sand above this half an inch in depth; over fifty larvae were tested, and it was found that with a few exceptions all went right to the bottom of the tubes to pupate; the exceptions reached only the top of the damp layer. Occasionally pupation occurred on a dressing applied to an animal's limb over a tumbu lesion.

#### EXPERIMENTS WITH PUPARIA.

*At room temperature.* On 16th February, 1923, nineteen puparia derived from the larvae of a wild rat were placed at room temperature; of these seventeen emerged either on 26th or 27th February, 1923. One pupa failed to give rise to an adult, and one adult died while emerging backwards; it may be noted that on several occasions the fly was thus inverted in the puparium. Newstead (1907) drew attention to this occurrence in the case of *Auchmeromyia luteola*.

*In the ice chest.* On 16th February, 1923, twelve pupae from the same source were placed in an ice chest. None of these had emerged by 9th March, 1923; they were then placed at room temperature, and of the twelve, nine emerged on 14th March, 1923; the other three failed to emerge by 10th April, 1923, when the experiment was terminated. The resistance of the fly in the pupal stage to cold is of interest, as is also the marked prolongation of the pupal stage under these circumstances.

*In Incubator at 37° C.* On 16th February, 1923, twelve pupae from the same source were placed in the incubator at 37° C.; on 2nd March, 1923, none had emerged; eight were removed from the incubator and placed at room temperature, four being left in the incubator. Of the eight removed, four were kept dry and four were kept moist. On 9th March, 1923, none of the twelve had emerged; the four remaining in the incubator were placed at room temperature, dry. On 10th April, 1923, none of the twelve had emerged and the experiment was terminated. The resistance of the fly in



the pupal stage to dry heat is therefore small. A similar observation has been made by Dove (1918) on the resistance to heat of the pupae of *Gastrophilus haemorrhoidalis*.

*Temperature and dryness.* In order to determine, if possible, whether the failure to emerge at 37° C. was due to the effect of temperature or dryness on the pupae, the following experiment was carried out with six pupae derived from a natural infection in a mongoose. Three pupae which had pupated on 19th and 20th March, 1923, were placed at room temperature in a calcium chloride desiccator. Three adults emerged on 1st April, 1923, from the three pupae.

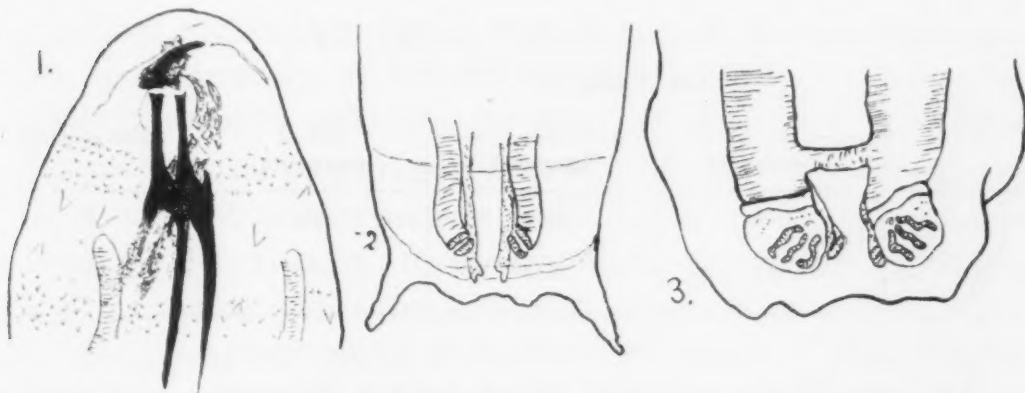
Three pupae which had pupated on 21st March, 1923, were placed in the incubator at 37° C., on 11th April, 1923, none had emerged; they were taken out and placed at room temperature; on 16th April, 1923, none had emerged and the experiment was stopped. So far as these experiments go, it appears that it is the height of the temperature and not the lack of moisture which prevents the development of *Cordylobia* at 37° C.

*METHOD OF EXIT OF FLY.* The puparium is broken at a short distance from the anterior end, and the whole cap or segments of it come off. The fly on emerging pushes vigorously through any resistant substance to escape; it was interesting to see that where puparia had been placed on a plug of wool half-way down a test-tube, plugged also at the mouth with wool, the flies which emerged most frequently went downwards through the wool on which the puparium was lying so that finally they arrived at the bottom of the test-tube. The newly emerged fly possesses great powers of forcing its way through such substances as sand, and cotton wool. As soon as it emerges, it sets about the operation of getting out of its immediate environment. The pressure exerted by the fly was very remarkable; in many cases two inches length of tight cotton-wool plug in the mouth of a test-tube being insufficient to prevent them escaping; in a few instances where the plugs were used, flies had flattened themselves between the plug and the glass to such an extent that they died, with the ptilinum extended in front of them and the wings still unexpanded. Dove (1918) carried out some experiments with *Gastrophilus haemorrhoidalis* puparia, which also showed the capability of the adults to push their way through



obstructing material. Thirty-two puparia were placed in moist loam at a depth of five inches; of these twenty-nine emerged normally, the adults pushing their way up to the surface.

**ECDYSIS.** The insect from the time it hatches out of the egg as a larva until it emerges from the puparium as an adult fly has undergone four ecdyses. The larva undergoes the first and second ecdyses in the tissues of the host, the first occurring from the second to the fourth days, and the second from the fifth to the sixth day. These dates were determined by removing larvae from their site in the skin of experimental animals at regular intervals after their first entrance, and examining them. The skin is cast from before backwards; the new stage emerging from the anterior end; we have been fortunate in finding larvae at various stages of the moulting process; for example, some specimens showed a condition in which



M.G.T.

FIG. 5. (1) First ecdysis, cephalic end. (2) First ecdysis, caudal end. (3) Second ecdysis, caudal end.

successive stages of the buccal armature were present together, while the posterior stigmata were still only in the earlier stage. Other specimens had the anterior portion of the cast skin loosened and crumpled backwards, while the posterior extremity of the cast was still firmly adherent, and showed the posterior stigmata of the successive stages co-existing (see fig. 5).

The third ecdysis occurs when the fly pupates, the larval skin not being got rid of, but remaining to form the puparium; the pupa which arises inside the puparium undergoes an ecdysis; this constitutes the fourth ecdysis. The pupal sheath cast off is found in the posterior end of the puparium when the fly has emerged.

Pupae removed from the puparium a little time before the time for the fly emerging were found to be enveloped sometimes completely and sometimes partially in the pupal sheath. In some cases it remained only as a delicate covering on the legs.

SIZE OF ADULTS. In the experiments related above, where certain pupae were subjected to the temperature of an ice chest, and in which the pupal period was thereby prolonged greatly, the size of the emerging flies was not appreciably affected. Of the flies which emerged in the experiment, twenty-two were measured: the males, ten in number, measured from 8 to 10.5 mm.; the females, twelve in number, measured from 8.5 to 10.5 mm. It is noteworthy that the size of the larva is no accurate guide to the size of the resulting puparium nor of the emerging adult; it is only a guide within rough limits. For example, two larvae gave the following figures:—

	Size of larva		Size of puparium	Size of adult
	Contracted	Extended		
1 ... ..	10.0	11.5	9.0	8.5
2 ... ..	9.5	11.0	10.0	10.5

The size of the emerging fly depended, however, on factors of which the degree of development of the larva is clearly one. Larvae which are removed early and which pupate give rise to flies which are small, while larvae which mature and pupate naturally give rise to large flies. In Plate XVI are shown two series, the first males and the second females, which demonstrate clearly the great variations in size which the fly may present.

FERTILISATION OF THE FEMALE. Copulation took place at once when recently emerged females were placed with wild male flies; it did not matter whether the females had fed or not; copulation lasted about two minutes and was repeated several times during the first day: after that it was not seen to occur; newly emerged males did not copulate readily.

## V. DEVELOPMENT OF *C. ANTHROPOPHAGA* Grün. IN ANIMALS

### (1) DURATION IN EXPERIMENTS

In the table given below are shown the various stages of the fly as it occurred in two of the experimental animals, a guinea-pig and a wild rat.

It is seen from the table that the development times in these cases were nearly the same, but the regularity with which the larvae in the rat completed the development was greater. As a rule, we found also that adults from rat puparia, as well as the puparia, were on the average somewhat larger than those from guinea-pigs.

### (2) PATHOGENICITY TO ANIMALS

IN NATURE. The animals most commonly found by us to be affected were wild rats, both brown and black; the feet, the genitals, the tail and the axillary region were chiefly involved where single larvae were present; in heavily infected animals any site was apparently suitable, including the nose. Dogs were also affected, and other animals more rarely. Lesions of the feet of sheep and goats were attributed by natives to the larva, but we did not find evidence of infection in those examined—about fifty. Various animals presented old scars and also suppurating sinuses which from the appearance might have contained larvae. The resulting lesions produced by the larvae during growth are illustrated by the case of a wild rat which had torn part of the skin off its abdomen in endeavouring to get rid of the larvae. As a rule, however, animals even with many larvae present either would not, or could not, get rid of them even when in quite accessible places. Several rats and a mongoose died apparently as the result of natural infection.

IN EXPERIMENTS. Guinea-pigs which were allowed to walk on infected sand soon showed signs of infection in the papule formation on the feet; by the third day the feet began to swell and the animals were seen biting them; in two days more great oedema of the feet was present, and in the oedematous skin the larvae could be seen; the posterior end was below the surface as a rule, and the circular aperture was smooth and polished on the margin; the

TABLE II.

Giving the development of *C. antropophaga* in Guinea-pig and Wild Rat.

Animal	Larva Number	Days in skin	Days to pupation	Days to emergence of adult	Remarks
Guinea-pig ...	1-6	3	...	...	Removed for experiment
	7	8	...	...	Did not pupate.
	8	8	10	22	
	9	8	11	22	
	10	8	11	...	Did not emerge.
	11	8	10	22	
	12	8	10	22	
	13	8	10	22	
	14	8	11	22	
	15	8	11	22	
	16	8	10	...	Did not emerge.
	17	8	12	23	
	18	8	10	22	
	19	8	...	...	Larva lost.
<i>R. rattus</i> ...	1	8	10	23	
	2	8	10	23	
	3	8	10	23	
	4	8	10	23	
	5	8	10	23	
	6	8	10	23	
	7	8	10	24	
	8	9	11	24	
	9	9	11	24	
	10	9	11	24	
	11	9	11	24	
	12	9	11	...	Pupa preserved.



posterior stigmata are easily seen with a hand-lens at this stage, and also the more striking tracheal tubes leading forward from them, which appear as two parallel silvery lines. The cavities occupied by the larvae are of some depth, because often the posterior end of the larvae is not flush with the skin surface, but lies several millimetres below this; as the mature larva may measure up to about 15 mm., it is seen that the larva may reach with its mouth parts a point about 2 cms. from the skin surface. One guinea-pig in this experiment died as the result of the infection; the larvae were found to have penetrated to the tendons of the feet; the overlying tissues were very greatly thickened owing to the oedema. In one guinea-pig where a larva had attacked the abdominal skin, it had penetrated so deeply as to cause a tumour pushing inwards the parietal peritoneum, which was congested deeply and thickened. Wild rats and small animals frequently died as the result of infection.

In sections cut through the larva in situ in the skin of animals, where no sepsis was present there was noted only oedematous thickening and moderate round-cell infiltration of the tissue surrounding the larval body.

### (3) THE ANIMAL HOSTS IN NATURE OF *C. ANTHROPOPHAGA* Grün.

From various sources, beginning at the early observations of Coquerel and Mondière (1862) and coming down to the present day, we have collected a list of animals in which larvae of *Cordylobia* have been found. In the notes of most observers the two animals which come first are man and dog, the other hosts appearing in the records of a more limited number of observers. Neave (1912-13) mentions that dogs suffer badly, and a case in a rat. It is a very natural thing that especial note should be made of conditions affecting man, and also that any condition affecting the dog should be the subject of remark, owing to the intimate association of this animal with man. It is this natural tendency which explains, we believe, the commonly held opinion that the dog is the usual host in nature of *Cordylobia*; of this belief we shall say something later. We have found the following animals recorded as hosts:—Man, dog, guinea-pig imported and locally bred, wild rat, various



monkeys, white rat, cat, wild cat, arvicanthus, squirrel, goat and antelope. Roubaud (1914) expresses doubts about the occurrence in goats and antelopes. A case of a mule infection was reported to us, but we had not the opportunity of investigating it. We have found larvae in addition in the mongoose and chimpanzee at Freetown.

#### (4) THE MAIN NATURAL RESERVOIR OF THE INFECTION

It is a matter of great importance to determine which animal forms in nature the reservoir in which the infection is maintained and from which man derives his infection. Le Dantec and Boyé (1904) wrote, 'Le chien est l'animal de choix pour la culture de la larve.' Roubaud was struck by the fact that dogs are so often affected, as reported by previous workers and also from his own observations. He writes, of dogs, 'Ce sont ces animaux qui constituent le reservoir permanent de la myiase furunculeuse,' and that there is a striking relationship between the human cases and the presence of dogs in the immediate vicinity of those men who are infected. More than this, he concludes that the rarity or absence of dogs in any region is one of the most immediate causes of the absence of the larva from that region. 'Il semble que l'abondance du ver dans un pays soit souvent fonction de celle de la population canine.' The logical deduction which Roubaud makes is in regard to the prophylaxis of Cordylobia Myiasis; as dogs are the natural reservoir, it is against the infection which exists in dogs that man must take action. The action recommended by him consists in the regular inspection of dogs at least once a week, the removal of larvae from them, and the destruction of the larvae. He anticipates considerable results from carrying out this procedure, 'En détruisant ainsi quantité de parasites, à la source même qui les entretient normalement, on arrivera nécessairement à faire disparaître la Cordylobia des endroits qu'elle infeste, ou tout au moins à la rendre extrêmement rare.' There is no doubt but that a careful attention to the expression and destruction of larvae in dogs will diminish the numbers of Cordylobia, and here we are in complete agreement with Roubaud. Where we differ is in regard to the question of dogs being the natural reservoir of the fly; we do not believe that the dog, easily and heavily infected as it doubtless often is, plays such an important part in the maintenance and spread of infection.

as to justify the optimism of Roubaud with regard to the results of his suggested method of prophylaxis. We found that in experimental trials, wild rats proved themselves more suitable hosts for the development of larvae than did even young dogs, that in nature wild rats were more frequently and severely infected than dogs, and finally we have been able to prove a close association between *Cordylobia* and wild rats, by finding in burrows of rats puparia of *Cordylobia* and rearing from them adults.

In one comparative experiment, using two pups and two medium-sized wild rats, twelve first instar larvae were allowed to penetrate the skin of the abdomen of each animal. Two larvae developed on pup number one, three on pup number two; on rat number one, eleven developed, and on rat number two, eight. The larvae from the dogs left their hosts on the tenth day, those from the rats left their hosts on the eighth day; the average size of the dog larvae was 12 mm. contracted and 14 mm. when extended, of the rat larvae was 12.5 mm. to 14.5 mm.

In nature, apart from large numbers of rats which had a few larvae in their tissues, we observed cases in which very severe infestation caused death. For example in a wild rat, *R. rattus*, nearly full grown, which was brought into the laboratory in a moribund state, there were present in various parts of its body forty-three larvae. The nose, the feet, the genitals, the tail and the general body surface were affected; the animal was in a very septic state and died soon after being brought in. Another rat presented no less than forty-one larvae, and here again the infestation resulted in death.

We have already seen that first instar larvae penetrate the skin of young rats more rapidly than that of any other animal tried; the larvae maintain themselves and develop in a higher percentage in rats than in dogs or guinea-pigs or any other animal used; the time taken by the larvae to develop in the tissues is shorter in rats than in dogs; the larvae which result from the rats are larger than from dogs. So far, then, as experiments are concerned, there is proof that the rat is a more suitable animal host for the larva than is the dog. In nature also we have found the rat more suitable.

We believe that Roubaud himself was aware of this high susceptibility of wild rats from the following three facts. The first

is, that he knew of the observation of Koch in East Africa reported by Dönitz; in East Africa, Koch found a disease in epizootic form which was killing wild rats; this disease on investigation proved not to be due to plague bacilli as first suspected, but to fly larvae present in the tissues; it was, indeed, this fly which Dönitz named *Cordylobia murium*, and which Roubaud is at some pains to prove is, in reality, *C. anthropophaga* affecting wild rats. The second fact is, that in discussing certain observations of Delanoe on *Cordylobia* in rodents, Roubaud says, 'Elles montrent que ce parasite peut trouver, en l'absence de l'homme ou de gros mammifères domestiques, son réservoir naturel chez de petits rongeurs, avec une électivité très marquée pour certaines espèces à l'exclusion des autres.' The third is, that he made the following experiments:—On page 141 of his work, his experiment B on development in the dog, resulted in twenty-two out of thirty larvae developing on the skin of a dog; he concludes, 'Le chien apparaît donc comme un hôte de choix pour l'évolution du ver du Cayor: c'est la confirmation de ce que l'on constate par l'observation naturelle.' There follows on this immediately, however, his experiment C, on development in the rat; in this twelve larvae developed out of twelve tried on the grey rat (*Mus microdon*), i.e., four larvae on each of three rats. Of this he says, 'La facilité avec laquelle le ver du Cayor évolue chez le rat, fait de cet animal l'hôte qui s'indique le mieux pour l'étude du parasite.' It is remarkable that in spite of the evidence which Roubaud himself produced in the experiments given above, he should have laid such undue emphasis on the part played by dogs in this disease in nature.

##### (5) TEMPERATURE OF ANIMAL HOST IN RELATION TO INFECTION

Roubaud (1914) observed that in certain species of vertebrates there was a more or less complete immunity towards the larvae. He considers that the development of the larvae is easy in proportion as the temperature of the animal host is low. He gives the following table of rectal temperatures in support of his thesis:—

Rat	...	...	...	...	...	36°5
Dog	...	...	...	...	...	38°5
Guinea-pig	...	...	...	...	...	39°5
Pig	...	...	...	...	...	39°5
Fowl	...	...	...	...	...	42°0

He found as we have seen that, in experimental work, the rat was a favourable animal, and that the fowl was useless for the development of larvae; we saw that he regards the dog as the natural reservoir of the infection. Man, however, he regards as secondary, 'L'homme ne représente certainement qu'un hôte accidentel, chez lequel l'évolution ne se fait pas toujours.' This statement does not accord with the theory that body temperature plays a part of paramount importance in this matter, as the rectal temperature of man, 37.2, is intermediate between that of the rat and that of the dog; from the point of view of rectal temperature man should form a very favourable host. Moreover, our experiments with guinea-pigs showed that they could often be readily and severely infected, and that the infection might even be fatal.

#### (6) DISCOVERY OF PUPARIA OF *CORDYLOBIA* IN NATURE

As a result of our laboratory experiments and the observations made on rats infected in nature, we concluded that rats are of great significance in the maintenance of the infection; the larvae found in rats must, we thought, emerge to pupate in the places where rats rested; it was decided, therefore, to investigate rat burrows and search for puparia. Although rats are common in Freetown, it proved no easy task to find rat burrows which were well defined, and which at the same time were situated so as to be capable of being dug out. Dr. W. Allan, W.A.M.S., the Medical Officer of Health for Freetown, kindly rendered us great assistance in this matter, and indicated two accessible rat burrows, one on open ground; the first burrow yielded puparia of *Cordylobia*; the soil at the entrance was carefully searched before digging was begun; the puparia were found in the first foot of the burrow proper, where the soil was light, dry and friable; from nine of these puparia which were still unopened, adult *Cordylobia* emerged in the laboratory. The second burrow was in the side of a laterite wall, and one puparium was found here, but the burrow could not be followed into the wall. In addition, puparia were found near the bungalow in rat holes among the rocks, one in each of two localities; from these adults emerged. Emphasis should be placed on the fact that along the river front at Freetown, and also along the small streams through the town, the occurrence of natural hiding places among



loose boulders, clefts in the rocks and unpointed walls, makes it possible for rats to exist easily without the necessity of making definite burrows. This fact will make the thorough survey of rat holes for *Cordylobia* a difficult operation, and will also naturally render any action against them prohibitive in cost. We are of opinion that these observations not only add one to the already long list of diseases for which the wild rat may be responsible, but also demonstrate that the prophylactic measures against dog infection suggested by Roubaud are unlikely to be as effective in eliminating either the fly or the disease caused by it as he anticipates.

#### VI. THE AGE INCIDENCE OF THE DISEASE IN NATURALLY INFECTED ANIMALS

It is common knowledge among natives of Sierra Leone that young children are more often affected by Myiasis due to *Cordylobia* than are grown persons; it is also known by them that young dogs are more frequently and more heavily infested than large dogs. They are so well aware of the fatal consequences which attend infection with the larvae of young pups, that in some places it is regarded as best to drown young pups when heavily infested. Maberley (1918) refers to Dr. K. K. Cross' observation, recorded in Sir Harry Johnston's book on British Central Africa, of maggots in native children—the whole side of a child riddled with holes. Marshall (1902) says that in Salisbury one baby had no less than sixty maggots extracted from it, and that there had been several cases in which babies had had a dozen or more. Grünberg (1903), quotes the observation of Steudel, who in Bagamoyo found larvae regularly on young dogs, but not on grown dogs; in these cases Steudel thinks that the fly seems to like to lay its young on the still soft and moist skin of the new born animal. Le Dantec and Boyé (1904) say 'young dogs above all are severely attacked, and one has sometimes to remove five or six larvae daily for several weeks in succession.' Smith (1908) says 'babies at breast and carried in a cloth on their mother's back are often affected. . . . Small pups suffer more than adult dogs and the larvae are all over them.' Roubaud (1914) mentions that he and Bouet observed numerous cases among dogs in Dahomey, and that at Khombole, even in the



dry season, he found young dogs infested; he adds ' Dans le Baol, j'ai observé de véritables nids de larves chez les petits chiens. Les adultes sont d'ordinaire beaucoup moins infestés.'

#### NON-DEVELOPMENT OF LARVAE IN SKIN OF LIVING ANIMALS

Although uninjured first instar larvae penetrate with great certainty into the skin of many species of living animal, even when the larvae are many days old, it does not follow that once established in the host tissues they will develop to maturity. This is a matter of considerable significance, and a few detailed observations may be given.

#### EXPERIMENTS ON HUMAN SKIN.

*Adult European.* 30th January, 1923. A larva 0.9 mm. long was placed on the back of the first phalanx of the little finger at 11.30 a.m.; the larva moved quickly into a wrinkle and it proceeded at once to penetrate, the mouth parts moving very rapidly making an entrance wound; the body pushed quickly into the aperture with a rippling movement, starting from about the ninth segment; no sensation whatever felt. 11.40 a.m.: First sensation felt when body of larva half concealed. 11.45 a.m.: Mouth parts ceased working for a minute; at this time only two posterior segments uncovered; the median mouth spine and sclerites plainly visible through a thin covering of cuticle. 11.50 a.m.: Whole larva concealed in tunnel of cuticle. 1.30 p.m.: Definite constant itching; redness around larva and swelling at situation of anterior end.

31st January, 1923. Definite papule formed; no irritation.

1st February, 1923. Papule larger; no itching, no irritation.

The after history of this larva, as also of one which penetrated the same day on the dorsum of the second finger first phalanx, close to a hair follicle, was that the papule gradually disappeared without further itching and discomfort. Similar experiments were subsequently performed on Europeans.

*Adult Africans (Young).* In two Africans, experiments were done with four larvae on the inner side of the upper arm.

The history of all these human experiments was similar; the larvae penetrated rapidly and caused irritation and papule formation. After three days they caused no more trouble and the

papules gradually disappeared. Roubaud applied two first instar larvae to his arm, and one to that of Dr. Bouet. The three larvae penetrated normally; as a result there was a partial development only, which went on for twenty-four hours. After this period no development occurred and the small lesions healed easily.

#### EXPERIMENTS ON ANIMAL SKIN.

*Guinea-pigs.* Eighteen larvae penetrated the skin of the feet of two guinea-pigs; of the eighteen only eight developed; these were removed on the eighth day.

*Chimpanzee.* Five larvae penetrated the skin of the forearm; none developed; in this case the animal was not prevented from scratching.

*Cercopithecus callitrichus.* Three larvae penetrated the skin of the tail, but none developed.

From these experiments the information was obtained that larvae which have succeeded in penetrating skin do not always develop; and that this lack of development could not be attributed in most cases to mechanical interference on the part of the animal.

#### VII. RELATIVE IMMUNITY OF OLDER ANIMALS

Numerous observers, as we have seen, have recognised the fact that young animals are more highly susceptible to *Cordylobia* infection than old animals. From what we know of the bionomics of the fly and the method by which first instar larvae gain access to the animal body, it is improbable that the comparative rarity of the disease among older animals is due to lack of opportunity of acquiring the infection. It appears clear that the failure of adult animals to show infection is due not so much to non-penetration of the larvae into their skin, as to non-development of larvae after entrance to the skin. The body is capable of resisting the process of development of the larvae but not the entrance of the larvae; the condition is one of relative immunity. If this relative immunity exists, and we have no doubt from observation and experiment that it does exist, we must endeavour to ascertain its nature. It is not an inborn hereditary immunity, because native children and the offspring of animals belonging to the country do not possess this

immunity. It is possible to suppose that it is an immunity which results from age alone, a form of immunity which it is easier to postulate than to prove. Evidence against its being an immunity which arises simply as a result of the maturity of the animal, is provided not only by the first, but by many subsequent observers. Coquerel and Mondière (1862) mention the case of an adult spaniel which was infested by a hundred larvae and died after some days; Bérenger-Féraud (1872) gives an instance of a spaniel which had seventy-eight larvae, while a pup of the same breed had three hundred, and died. Again, adult immigrants to West Africa, involving many nationalities, including Europeans and, as mentioned by Blenkinsop (1908), West Indian troops, are affected by the larvae. It appears improbable that age alone confers immunity. There remains immunity acquired as the result of previous attacks of the larvae, and this we believe to be the true cause of the fact that older animals are less frequently infected than are young ones. Experimental evidence of such an acquired immunity is naturally not easy to produce, as the animals with which experiments are carried out in Tropical Africa are almost certainly partially immune; the repeated entry of larvae to the skin and their subsequent destruction by scratching and rubbing must constantly go on. The following human observations may, however, be quoted. An adult European in Sierra Leone suffered from a natural infection, nine larvae developing in the skin; thereafter he proved resistant to infection at several attempts, as shown below:—

TABLE III.

Showing the result of attempts to infect a human adult with *Cordylobia* larvae.

Date		Number of larvae which penetrated skin		Site in body	Result
September, 1922	...	Natural infection ...	9	Larvae in upper arm	Removed at 6-8 mm. long
January 30, 1923	...	Experimental infection	2	Fingers ...	Death of larvae.
February 18, 1923	...	Experimental infection	4	Arm ...	Death of larvae.
March 7, 1923 ...	...	Experimental infection	4	Arm ...	Death of larvae.
March 30, 1923	...	Experimental infection	6	Arm ...	Death of larvae.
April 6, 1923 ...	...	Experimental infection	12	Arm ...	Death of larvae.
April 10, 1923 ...	...	Experimental infection	4	Arm ...	Death of larvae.
April 23, 1923 ...	...	Experimental infection	5	Arm ...	Death of larvae.

Certain animals gave suggestive results. Two dogs which had been infected on the abdomen with partial success, resisted infection at a subsequent date with eighteen larvae each, all of which penetrated. Into the skin of two guinea-pigs which had recovered from an experimental infection there penetrated on 7th March, 1923, four and five larvae respectively; on 15th March, 1923, six larvae; on 20th March, 1923, six larvae; on 23rd March, 1923, six larvae; on 28th March, 1923, four larvae. Although the larvae developed for a period varying from a few hours to two days, in no case did they develop beyond the first instar. A monkey previously naturally infected on the root of the tail received on the perineum on 2nd April, 1923, nine larvae; on 8th April, 1923, six larvae; on 10th April, 1923, six larvae; no development occurred beyond a papule formation which resolved on or before the third day. These instances appear to show that there is an acquired immunity against *C. anthropophaga*.

On the other hand, we encountered certain paradoxical results, as for example, the following:—On 7th March, 1923, a Creole youth received four larvae in the upper arm; development to papules only; on 8th April, 1923, a single larva penetrated and this developed normally. Again, a small dog which had had infection and had thereafter proved resistant to several infections, at the third attempt received ten larvae on 10th April, 1923; of these three developed normally. In each of these two cases anthelmintics had been administered, to the human case betanaphthol before the last infection, and to the dog carbon tetrachloride on the same day as the infection; but whether this fact is merely coincidence it is not at present possible to say.

Against the immunity theory we have also the fact that large rats often present infection; we must conclude from this, either that such rats had not acquired immunity in their early days, or else that their immunity was broken down by some cause late in life. It is not possible, with our present knowledge, to explain why this immunity, as do all forms of immunity, breaks down on some occasions. The broad conclusion, nevertheless, is that there exists among adult animals an immunity against *Cordylobia* larvae, and we believe that the foregoing experiments point to its being an immunity acquired through previous attacks of the larvae, whether



these developed or were destroyed before developing by mechanical interference on the part of the host.

#### DURATION OF IMMUNITY

So far as we are aware, there are few facts available as yet upon this point; the following observation of Heckenroth and Blanchard (1913) has some significance and deserves mention. A European in the Congo had a fox terrier which acquired infection in October, 1911; the owner got infected in November, 1911. The owner and the dog left Africa and returned in over a year. In January, 1913, the dog became infected, and in February, 1913, the owner became infected; the immunity if established by the first attack, did not persist for much over a year at the most.

#### CUTANEOUS REACTION

In the human case, where repeated attempts were made at infection, there was a severe local reaction at each of the last six attempts; the penetration of the larvae was accompanied by great itching and was followed within a few minutes by remarkable local signs. At the point of entrance of each larva a white bleb formed and spread rapidly in all directions; the larvae were placed on an area of about two inches by three, and yet in all cases within fifty minutes the white urticarial wheals had coalesced and produced an irregular swelling about three inches in diameter and raised in the centre about a quarter of an inch; around the white area was a zone of deep congestion which faded away at the margins. The rather tense white swelling stood out in a striking manner against the red background; very considerable itching was felt over the whole area affected, while at the same time a slight pricking sensation caused by the larvae boring was experienced. By next day the swelling, redness, and to a great extent the itching, had subsided.

Hadwen and Bruce (1917) made observations on Anaphylaxis, in cattle and sheep, produced by the larvae of *Hypoderma bovis*, *H. lineatum*, and *Oestrus ovis*. Intravenous injection of larval extracts in saline solution produced death rapidly in some animals, severe reactions in others, while in some it produced no ill-effect. The preliminary sensitisation was not experimental but natural, and



was attributed to the excretions of the larvae; in one case there was evidence of the sensitiveness being inherited. Anaphylaxis was only found to occur when larvae were broken in an animal, or when a dose had been injected. An ocular reaction occurred in sensitive animals when a drop of juice from a larva was placed on the conjunctiva. The cutaneous reaction in our case, while apparently anaphylactic in nature, resulted not as in Hadwen and Bruce's experiments from the injection of extracts, but from natural penetration of larvae into the skin, the larvae being uninjured.

These observations which we have made on immunity appear to us of great importance, not only in so far as concerns *Cordylobia* Myiasis, but still more in relation to the Myiasis caused by *Dermatobia* and *Hypoderma*, in which cases enormous loss is suffered year after year on account of damage to hides. The development of some method of immunizing cattle against the attacks of the larvae would result in a great increase of value of hides from countries which are affected by such larvae. The remarkable thing is that in South America, in the midst of a country in which cattle are severely affected by the larvae of *Dermatobia*, there actually exists a breed of cattle—Antioquia—which enjoys a relative immunity. We are indebted to Mr. M. T. Dawe, Commissioner of Lands and Forests in Sierra Leone, for showing us his photographs of this breed and for much interesting information concerning it. We feel convinced that a further study of this subject, with a view to discovering some means of artificially producing a definite immunity would well repay the trouble and expense involved.

#### VIII. SEASONAL INCIDENCE OF *C. ANTHROPOPHAGA* Grün.

The consensus of opinion of previous observers appears to be that the wet season is the season of prevalence of infection with *Cordylobia* larvae. Coquerel and Mondière (1862) stated that in the month of July, in Senegal, after the commencement of the rains, many cases of these larval parasites occurred. Le Dantec and Boyé (1904) say that in French Guinea, the adult appears in the beginning of the wet season, disappears abruptly in October, only to reappear next year with the first rains. Rodhain and Bequaert

(1913) mention a series of animals which are affected with larvae at Katanga, all in the wet season. Roubaud (1914) refers to Bérenger-Féraud's observation that in human beings this form of Myiasis occurs in July. Howard (1912-13) says that the larva is very abundant during certain seasons in the Transvaal. The infection of human beings is considered by Roubaud to result from close association of dogs with men, the fly being primarily attracted by the dogs. Our experience as regards adult flies is not in agreement with that of Le Dantec and Boyé; during the dry season, as we have shown, the adult fly is not rare in Freetown; we captured over a hundred wild specimens, both male and female; fertilization and oviposition occurred constantly during the dry season. There does not seem to be any reason why infection of man should not occur in this season if the theory that dogs are the attraction which brings the fly near man, and form the main reservoir, were correct. That these flies were not, in this case, attracted by dogs is evident, because there was no dog present in the bungalow; it was equally clear that they were not attracted by the latrines, as in no case was a fly ever captured or seen there; nor were they attracted by the clothes accumulating for the laundry, which they were never seen to approach. They appeared not to have come in to lay their eggs, because males as well as females came in, in about equal numbers and because no female which was captured was actually ready to lay eggs when it came indoors. The correct explanation probably is, in accordance with the experimental evidence on the effects of sunlight, that the flies were simply taking shelter from the heat of the sun; we have pointed out that on dull days they did not come in. The source from which these flies came was, we think, the rat holes in the rocks adjacent to the bungalow, where, as we have pointed out, puparia were found. Rats captured near the bungalow were frequently infected, and sometimes heavily.

It might be argued that, although in this case the flies were not attracted by the presence of dogs, if dogs had been present they would have induced the flies to oviposit in the house; that might be so, but if it were the case, how can we explain the absence of human Myiasis at this time of the year in houses where dogs are kept at all seasons of the year. The fact that there is a definite

wet seasonal incidence of Myiasis in man and also dogs points to some factor at work which is independent of the presence or absence of dogs.

One explanation which suggests itself arises out of our discovery that the wild rat is the chief natural reservoir of the fly; this explanation is, that the seasonal incidence in man and also in dogs is dependent on the seasonal habits of the rat. It is commonly known that in the wet season rats congregate more closely in the neighbourhood of human habitations; this movement is due possibly to the flooding out of their burrows; this theory involves that *Cordylobia* moves with the rat, and so is brought into close association with human habitations. Another explanation is that it is simply the desire to escape from wet which makes the fly lay its eggs indoors in the wet season.

The flies came in freely, in our experience, during the dry season, but not with the idea of ovipositing; their normal place for ovipositing in the dry season is not indoors. We have seen, however, that in experiments the fly avoids wet sand and will not lay her eggs there, she will rather even lay them on cotton wool and cloth, and this was clearly done in the case of natural infection mentioned below. This fact alone might account sufficiently for the increase in human Myiasis in the rains, and also to a less extent for the increase in Myiasis of domestic animals. It is probable, however, that both factors are at work.

#### IX. MODE OF INFECTION OF MAN

The earlier erroneous ideas that the fly lays larvae or eggs in the skin of man, or that it attaches its eggs to hairs, have already been referred to. The method of infection in man is by the penetration of the first instar larvae into the skin; for this the larvae must effect contact with the skin. It is clear that there are very many ways in which such necessary contact may be brought about. It is possible, for example, that where soil or sand, especially if contaminated, is used in latrines, the female fly may deposit her eggs in the sand box; if in the act of using the sand some of it is spilt on the seat of the latrine and first instar larvae

are present in the sand, the next person to use the latrine will almost certainly have the larvae penetrate the skin. Again, if flies are hard pressed, they will lay their eggs on clean clothes, and when the larvae hatch out, if the clothes are put on, infection will occur. An interesting case is that reported to us by Dr. Wright, of Freetown, a most accurate observer, who has great experience of this disease; he was called to see a patient who had injured his shoulder; he wished to put it up at once, and for a bandage was provided with a window curtain which had been washed and ironed and had been lying in the house for some time. In a few days, only under the curtain, there developed on the patient's thorax, back and front, and on the arm, thirty-eight larvae of *Cordylobia*; the other curtain which had been lying underneath the first was examined carefully by us, but no eggs or larvae were present; there appears here the strongest evidence of the infection from clean cloth. The probability of the larvae derived from eggs laid on dirty clothing surviving the washing, exposure to the sun to dry, and subsequent ironing appears very small. Again, in view of the adult fly's dread of the bright sun and the lethal effect which this produces on the fly, it appears improbable that clothes hung up on lines to dry in the sun would have eggs deposited on them; if the clothes were laid on the ground to dry they might pick up larvae easily from the soil, but would not be so likely to pick up eggs which are lying slightly below the surface. In the houses of natives, the occupants could obtain infection by lying on infected soil, and also in any of the previously mentioned ways.

## X. SYMPTOMATOLOGY

### IN ANIMALS

The presence of one or two larvae produces little obvious distress even in small animals; when the larvae are numerous, however, there is very considerable irritation, and the obvious illness of the animal results chiefly from septic absorption combined with loss of sleep; the appetite diminishes, and the animal loses weight. Where larvae are single there is little to note beyond the localized small tumour; where larvae are close together great swelling and oedema occur, and the tissues intervening between larvae become



sloughing and gangrenous; the removal of individual larvae, then, is difficult, as the whole area of skin surrounding it may come off with them. The larvae are not always confined to the true skin, but often penetrate into the deeper tissues; in the case of some of the guinea-pigs, as we have stated, they exposed the tendons of the feet. In the abdominal wall the parietal peritoneum may be involved, giving rise to rigidity of the abdominal muscles, retraction and tenderness. In certain regions the presence of larvae produces more serious lesions than in others; the feet and scrotum easily become gangrenous; the case of one pup which became blind of an eye was reported to us; the larva had penetrated the skin before the eyes were open.

#### IN MAN

The actual penetration of the first instar larva into the skin is hardly noticeable; in some cases, like the one referred to in a previous section, an intense cutaneous reaction occurs. Roubaud observed a similar reaction in his own case, and accounted for it by saying that the larva had become contaminated from the soil in which it was. It appears to us more probable that this is a body reaction in response to the presence of a specific substance produced by the larva, possibly of a salivary nature, and that it has some connection with the marked condition of immunity which existed in one of our human cases. Such a reaction was not observed by us in the case of another European, recently arrived in this country; in this case larvae of the same batch introduced themselves into the skin without reaction and proceeded to develop. The larva developing in man is felt during the first two days or so, causing slight itching or pricking at intervals; the symptoms and signs are easily overlooked. The papule which forms, increases in size and becomes red; there is then a more or less complete cessation of symptoms for several days possibly, although the furuncular swelling increases. Then the symptoms recur with greater severity; the pain increases and becomes so sharp as to interfere with sleep. The larva becomes very active at intervals and can be seen clearly retracting into the cavity and then pushing against the margins of the aperture in the skin to increase its size. Much serous fluid may exude at this time; the skin and subcutaneous tissues have meantime



become much indurated and the area round the aperture is deeply coloured; tenderness on pressure exists; the lesion resembles a boil, for which it is frequently mistaken. Gland enlargement may occur and general symptoms, malaise and febrile reaction. The development was slow in human beings observed; in one case a larva removed on the fifteenth day in the third instar measured only 9 mm. The stage at which larvae are usually brought under notice by human beings is after the third stage has been reached, at which time the larva, in enlarging the entrance aperture preparatory to making its exit from the skin, exercises considerable force. The cavity formation is out of proportion to the size of the larva, and it appears as if the larva produced a lytic action on the tissues near its head end; the clear fluid which comes from the cavity at intervals is sometimes stained with blood and also with faeces of the larva. On removal of the larva the symptoms disappear, and healing usually occurs readily.

Nagel (1897) observed larvae in his skin in East Africa for a period of four weeks; but the record is not complete.

#### XI. TREATMENT

Various methods of treatment were tried experimentally, such as the effect of tobacco smoke, insufflation of calomel powder, French chalk, dropping on tobacco juice, chloroform water, phenol solution and cresol solution in 5 per cent. strength, application of vaseline, palm oil and liquid paraffin. Simple expression was effective in removing the larvae, but often painful; removal by fine forceps was easy in the later stages if the aperture was large. Of these various methods the one finally adopted, especially for use with small larvae which cannot be removed easily with forceps, was the application of liquid paraffin and subsequent expression. Blenkinsop (1908) records the effect of a plaster of sugar and soap in causing larvae to emerge, owing to the blocking of the posterior spiracles. The natives of Sierra Leone use palm oil, the pericarp oil of *Elaeis guineensis*, with the same object; they say, however, that the Tumbu comes out at night to feed on the oil. Palm oil was tried, but was discarded owing to the colour; in its stead liquid

paraffin was used, and acted admirably even with the very small larvae. A film of paraffin is placed over the opening in the skin, any scab being first gently removed; at once the posterior end of the larva begins to come out; the film is then thickened by adding paraffin drop by drop; the larva in its efforts to reach the surface of the film makes greater movements out and in; in doing so it lubricates itself and the walls of the cavity; the superfluous paraffin is wiped off, and the two thumbs are placed a little distance on each side of the aperture and pressure inwards and downwards applied; the larva comes out slowly at first, later with more rapid movement. The larva should be destroyed. After extraction of the larva and healing of the wound, a mark remains for a long time; Fülleborn (1908) could still after ten years see the marks left on the skin.

## XII. PROPHYLAXIS

Adults should be looked for daily in houses on the ceiling of rooms and verandah, during the sunny hours of the day; any fly present should be captured in a collecting net and destroyed. All latrines should be fly-proof.

Sand and soil used for the latrine may be heated in a kerosene tin for some time before being placed in the latrine box, in this way eggs and larvae are destroyed.

In affected districts the weekly examination of domestic animals should be carefully done, and any larvae found, expressed and killed; it is most important to destroy the larvae.

Rats should be eradicated as far as is possible from houses and compounds. Those captured should be destroyed by burning before the larvae leave them.

In cases of small boils where there is doubt as to the presence of a larva, the application of a drop of liquid paraffin will cause the hind end of the larva to move actively; in any case liquid paraffin will be of great assistance in removing larvae with as little pain as possible.

Clothes lying exposed are a source of danger; it is especially underclothes and bed linen which are apt to carry infection to man; a very certain method of prevention here is to have all the clothes

ironed after washing and drying, and to store them immediately in covered receptacles.

It is advisable to have such clothes washed in the compound, and after ironing kept in drawers or suitable covered boxes; this simple precaution will prevent flies laying on them.

### XIII. COMPARISON BETWEEN *CORDYLOBIA ANTHROPOPHAGA* AND SOME OTHER MYIASIS-PRODUCING FLIES

Myiasis is a wide term embracing parasitism of very varying degrees, and involving widely different parts of the body. We shall confine our attention here to those forms of Myiasis caused by flies which in the first instar have been proved, or appear to be capable of penetrating the unbroken skin.

#### (1) *BOOPONUS INTONSUS*, Aldrich

Woodworth and Ashcraft (1923) describe a condition of Myiasis affecting the feet of carabaos and bullocks in the Philippine Islands; larvae were reared and adults bred and forwarded to Aldrich. Aldrich (1923) from three females sent to him created the new genus *Booponus* with the species *B. intonsus*. He refers to the close similarity of the adult to *Cordylobia*. The description of Woodworth and Ashcraft deals with two larval stages, but the illustration of the young larva shows that it is not like the first instar larva of *Cordylobia*; rather it resembles closely the second instar of this fly, not only in the appearance of the posterior spiracles but also in the very large and dark spines irregularly distributed over the cuticle.

It is surmised, but not proved, that the first instar larva which arises from eggs attached to hairs is capable of producing Myiasis by penetrating unbroken skin; if it appears later that this is so, we should expect, on analogy, that a first stage larva having a buccal spine will be found.

#### (2) *WOHLFAHRTIA VIGIL*, Walker

Walker (1920) described two cases of cutaneous Myiasis in infants due to larvae of this fly; evidence was given of a clinical character that these larvae appeared to have entered the unbroken

skin. Again Walker (1922) gives details of another case and also descriptions of the first instar; he says 'the median or labral hook arises from a slightly divided base, immediately in front of the pharyngeal sclerites, and is strongly decurved, the pointed apex projecting slightly from the front part of the oral aperture in the usual position.' The illustration shows well this curved hook; this character differs from the median spine in *Cordylobia* first instar larvae; the appearance of this apparatus in *Wohlfahrtia vigil* does not suggest that the mode of entering unbroken skin can be the same as in *Cordylobia*; it is possible that when skin penetration experiments are carried out, it may be found that this larva, if it can in fact penetrate healthy skin, does so by digging at once deeply into the tissue, and not, as in *Cordylobia*, by raising over its dorsal surface a thin layer of cuticle.

(3) *HYFODERMA* spp.

Laake (1921) gives an account of the anatomy of the mouth apparatus of *H. bovis* and *H. lineatum*; he refers to the fact that Riley (1892) first described the real first stage larva of *H. lineatum* which he obtained from the egg before hatching; he mentions that Gläser (1914) and Carpenter, Hewitt, and Reddin (1914) first observed the first stage larva of *H. bovis* outside of the egg. Laake in his description and drawings shows that the median mouth spine in these larvae is retained not only during the first instar but also actually during the second and third instars, during which the larva is passing through the tissues, and is not cast off until the larva reaches the back of the host. It is interesting to note that the ventral curvature of the spine is relatively slight compared with that depicted by Walker for *W. vigil*, and resembles more the condition present in *Cordylobia*.

(4) *DERMATOBIA CYANIVENTRIS*

Surcouf (1913) figures the first instar larva of this fly; the larva which is able to penetrate the unbroken skin is possessed of a buccal armature closely resembling that of *Cordylobia*; the large forwardly directed cuticular spines on the posterior segments which we drew attention to in *Cordylobia*, exist in this larva also.



## XIV. SUMMARY

1. The morphology and bionomics of *Cordylobia anthropophaga*, Grün., have been studied in some detail during the dry season 1922-23, in Freetown, Sierra Leone.

2. Certain new facts as regards the habits of the adult, its method of oviposition and the number of eggs laid by it are recorded.

3. In the first larval stage also, attention is drawn to certain morphological peculiarities, both in the buccal armature and in the cutaneous spinulation, which appear to have a direct and intimate connection with the process of skin penetration.

4. A direct association between *Cordylobia* and wild rats, which was suggested by field observation and laboratory experiment, has been proved to exist by the discovery of puparia of this fly in the burrow of wild rats.

5. Evidence is produced which appears to incriminate the wild rat as the main reservoir of the infection in nature, and to associate these rodents with such seasonal incidence of the disease as exists.

6. Numerous experiments were carried out both in man and animals, which add considerably to the knowledge of the mechanism of infection, pathogenicity and prophylaxis.

7. An immunity has been proved experimentally to develop against attacks of the larvae, not only in man but in animals.

8. The development of such an immunity in the case of cattle in similar forms of Myiasis is considered a possibility and worthy of investigation.

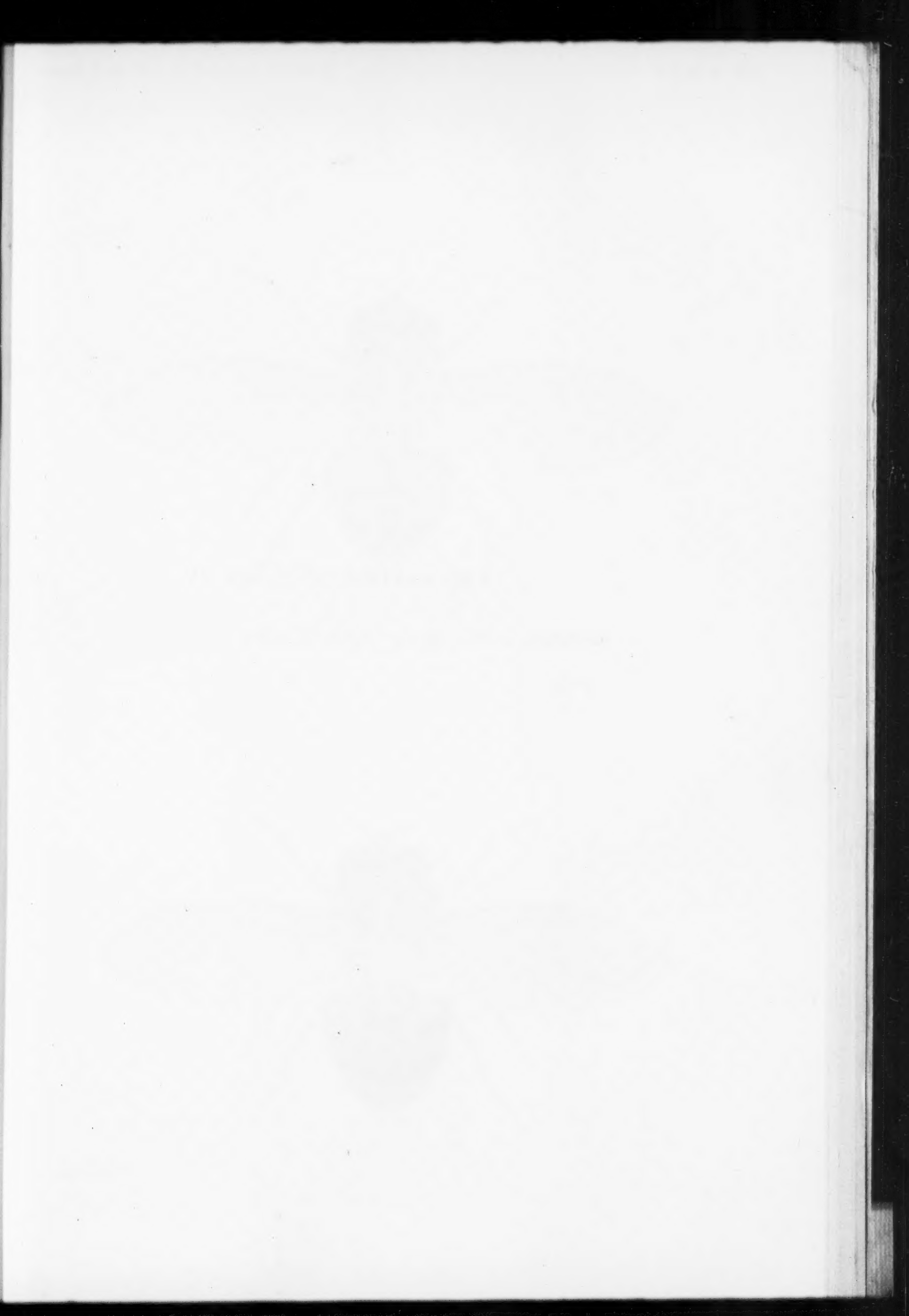
9. A comparison is made between *Cordylobia* and some other flies which cause cutaneous Myiasis.

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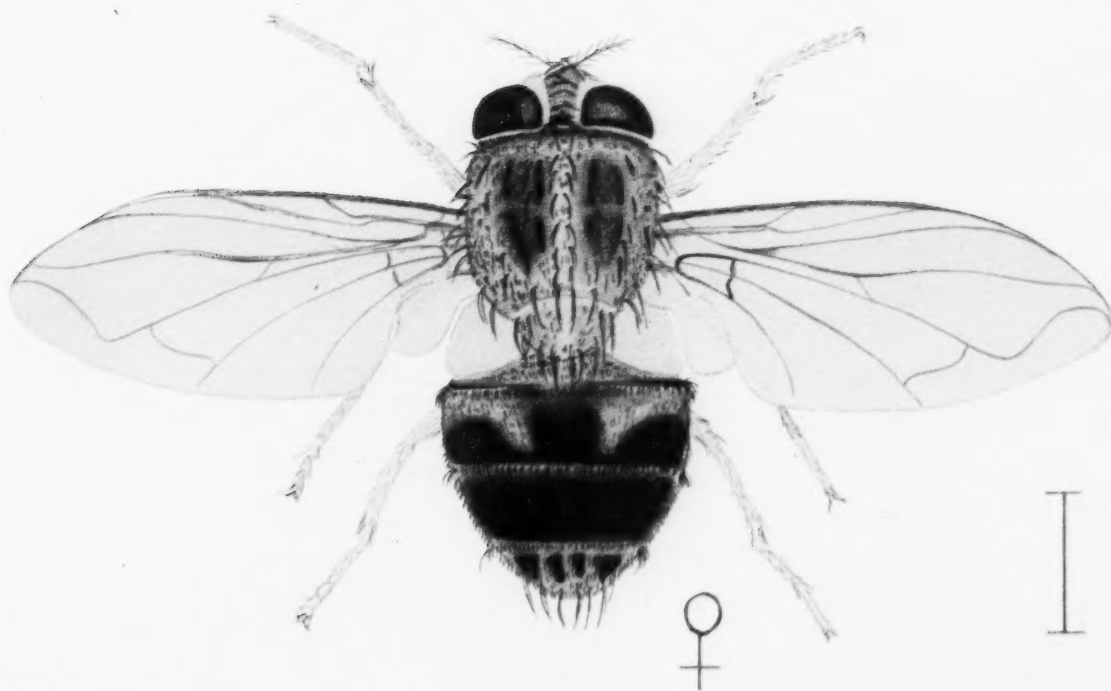
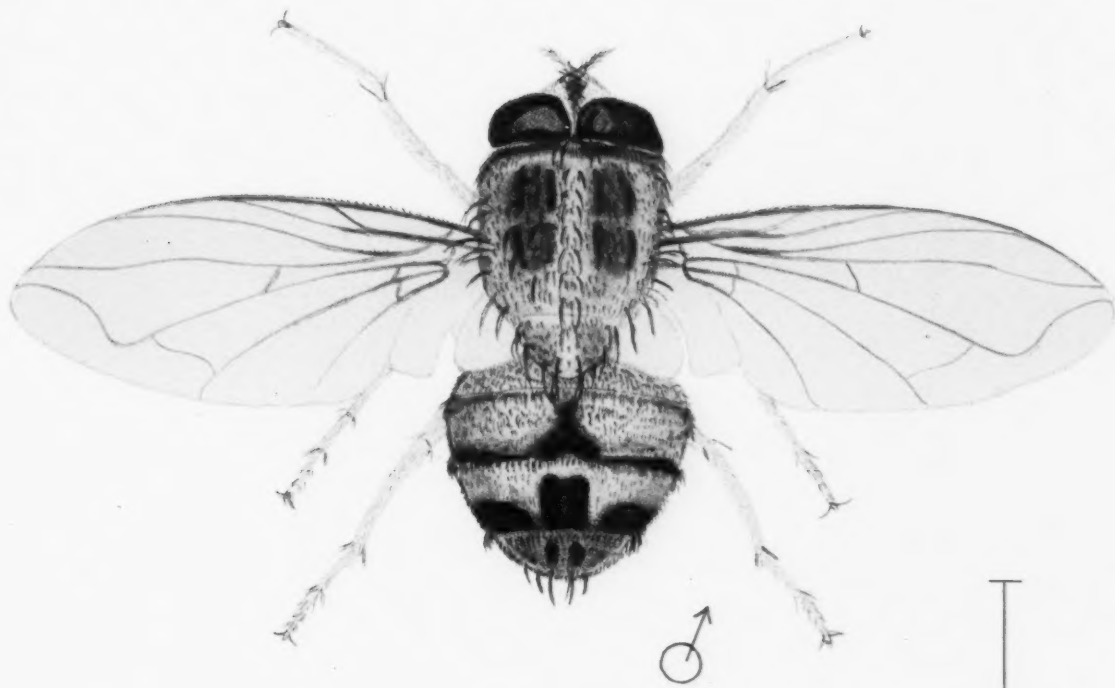


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## EXPLANATION OF PLATE XV

*Cordylobia anthropophaga*. Adult ♂ and ♀.



del. M.G.T.

## EXPLANATION OF PLATE XVI

*Cordylobia anthropophaga*. Photograph of series of adults and puparia.





*Photo. by M. Brown*

## EXPLANATION OF PLATE XVII

Photograph of naturally infected rats (shaved with Barium depilatory powder).



## EXPLANATION OF PLATE XVIII

*Cordylobia anthropophaga*. Photograph of second instar larva in the tissues of a guinea-pig.  $\times 8$ .



*Photo. by M. Brown*





# THE TRANSMISSION OF *T. CONGOLENSE* BY *GLOSSINA* *PALPALIS*

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## I. INTRODUCTION

Representatives of the group of trypanosomes to which *T. congolense* and *T. nanum* belong are widespread throughout Africa. They are common alike in game and in stock. To the game they are, apparently, harmless, but as parasites of domestic animals they constitute a factor of considerable economic importance.

The trypanosomes of this group are well adapted to rapid propagation in nature. For indirect or cyclical transmission they depend, so far as is known, entirely on the *Glossinae*; and, though less common than *T. vivax* and *T. uniforme* in wild game-tsetses, they are considerably more prevalent in the fly than the polymorphic trypanosomes of the *T. brucei* group. In all species of *Glossinae* in which cyclical development of the *T. congolense* group takes place, the flagellates multiply first in the gut of the fly, and later on take up their 'anterior station' in the labrum and hypopharynx of the proboscis.

In addition to cyclical transmission, they are readily conveyed

from mammal to mammal by the direct method—that is, without any alternation of generations. It is probable that most, if not all, the mammalian trypanosomes can, on occasion, avail themselves of direct transmission, but it is doubtful whether any other group of the *Glossina*-carried organisms relies to the same extent on this method in nature.

The so-called species contained within this group have been arbitrarily separated on account of differences in their behaviour in certain mammals. The *nanum*-form cannot infect dogs and monkeys and the small laboratory animals, while the *congolense*-form can; otherwise they are indistinguishable. Both these two forms of the trypanosome have been recovered from game and from wild fly, so that this idiosyncrasy may, to a slight extent, influence the distribution of the parasites in certain regions. But to allow to physiological variations of this kind the importance attaching to a specific character, is, as Yorke and Blacklock (1914) have pointed out, unjustifiable; and, as a matter of fact, strains whose virulence is intermediate between these two extremes do occur in nature. The group should, therefore, be regarded as a collection of strains whose morphology and behaviour in the insect intermediary are uniform and constant, but which vary in their relation to certain mammalian hosts. It has been shown elsewhere by laboratory experiments, that the effect of long-continued direct transmission on a strain of *T. brucei* may be to enhance its virulence in mammals, and, at the same time, to eliminate the power of developing cyclically in *Glossinae*. The same principles probably apply to the group we are considering. There is, indeed, field-evidence to show that some of the more virulent strains of *T. congolense* rely largely, if not solely, on direct transmission, for their passage from mammal to mammal. Whether such strains retain the power of cyclical development in *Glossinae* has, however, not yet been determined.

The gregarious habits of domestic stock afford excellent opportunities for the spread of their trypanosomes by direct transmission, by *Stomoxys* and the other species of biting flies that, in Central Africa, swarm around the unfortunate animals. Among the game this method functions probably much less commonly, although with certain species such as elephant, buffalo and eland the requisite conditions will doubtless often occur.

However this may be, there is an essential difference between game and stock in their relation to these parasites. For while the game is not, so far as is known, in any way inconvenienced even by double infections of the trypanosomes indigenous to its habitat—whether directly or cyclically transmitted—cattle, as a rule, cannot survive even brief contact with the game-tsetse, and are particularly sensitive to strains of *T. congolense* in non-tsetse areas. In localities where game-tsetse are present and game plentiful, a certain percentage of the wild fly will always be found to carry the developmental forms of the proboscis-and-gut group of trypanosomes. In *G. palpalis* areas, however, matters are different. The commonest flagellate found in this fly in nature is probably *T. grayi*. Of the mammalian trypanosomes, the proboscis-only group is most commonly found, represented by *T. vivax* and *T. uniforme*; the gut-and-gland group, usually in the form of *T. gambiense*, is also of common occurrence; but the proboscis-and-gut group is distinguished by the comparative rarity with which it develops in wild *G. palpalis*.

So far as I can determine, three instances are recorded of the occurrence of developmental stages of the proboscis-and-gut group in wild *G. palpalis*. These are as follows:—Warrington Yorke and Blacklock (1915), investigating an epidemic of cattle trypanosomiasis in Sierra Leone, found nineteen out of one hundred and forty-seven cattle infected with trypanosomes; *T. congolense* was found in sixteen, *T. vivax* in seven, and *T. gambiense* in one. In the course of the dissection of four hundred wild *G. palpalis* caught in the same neighbourhood, proboscis-only infections were found in fifteen flies, gut-only in two, and gut-and-proboscis in four.

Macfie (1915) dissected seventy-five wild *G. palpalis* from near Accra, and found eleven infected with flagellates—three gut-and-gland, three proboscis-only, and one proboscis-and-gut. In the review of these experiments, there follows the seemingly contradictory comment that 'none of these infections resembled stages in the development of *T. congolense*.' Possibly the flagellates in the proboscis of the single gut-and-proboscis fly were not fixed, and, in consequence, were not regarded as true proboscis-forms.

The third instance occurred in my own work (1916) in the Northern Province of Uganda, where, in two areas, proboscis-

and-gut infections of wild *G. palpalis* were found, amounting in one case to 1·4 per cent. and in the other to 5·2 per cent. of the flies dissected. Allowing for the possible occurrence in some of these flies of double infections, the last figure is sufficiently high to prove conclusively that, in this area, the *congolense-nanum* type of trypanosome is carried cyclically by *G. palpalis*.

In the face of these examples it is the more remarkable that no instance has hitherto been recorded of the occurrence of this group of trypanosomes in the wild *G. palpalis* of the Victoria Nyanza. At many points along the shores of this great lake, cattle came in contact with this tsetse; and there are long stretches of shore in Busoga where game and *G. pallidipes* occur immediately behind the *G. palpalis* shelter, and where the latter fly has plenty of opportunity for picking up parasites of the *T. congolense* group. *T. vivax*, *T. uniforme*, and a member of the gut-and-gland group are all found in these lakeshore *G. palpalis*. During the fourteen odd years that have elapsed since the depopulation of the Sesse Islands, the Situtunga have multiplied enormously, and the tsetses on these Islands have come to rely on the antelope to a great extent for food. The three trypanosome species just mentioned, are common in these Situtunga, but on no occasion has the *T. congolense* type been found in their blood. Apparently *G. palpalis*, in its natural environment, is not, as a rule, suited to the cyclical transmission of these trypanosomes.

A possible explanation of the absence of the proboscis-and-gut group from the fly on the Victoria Nyanza is, that the strains with which the fly come in contact are directly transmitted strains, which have lost the power of cyclical development in *Glossinae*. But this explanation alone is inadequate to account for the absence of these parasites from this huge fly area.

## II. PREVIOUS WORK ON THE TRANSMISSION OF THE PROBOSCIS-AND-GUT GROUP OF TRYPANOSOMES BY *G. PALPALIS*

The Royal Society's Commission in Uganda (1910) experimented with wild and with laboratory-bred flies. Two successful transmissions were reported. In one experiment, with wild *G. palpalis*, the flies became infective to a clean animal twenty-one



days after the first infecting feed; in the other, in which laboratory-bred flies were employed, the flies were infective on the fourteenth day of the experiment. The authors remark that these last experiments were open to fallacy, as an epidemic of trypanosomiasis, due to a member of the 'proboscis-and-gut' group, was occurring at the time in the neighbourhood of the laboratory. I think it probable that the same explanation applies to the wild fly transmission also. No positive flies were found on dissection of the flies in these experiments, and at the time, it was not known that the developmental cycle of *T. congolense* included an invasion of the proboscis of the fly. Subsequent experience has shown it to be improbable that the trypanosomes of this group can complete their cycle in *G. palpalis* in such a short time as twenty-one days.

Of the attempts by the same Commission to transmit *T. nanum* by *G. palpalis*, we read that the only experiment attempted was 'unsatisfactory, as trypanosomes appeared in the first healthy goat a few days after the fly had fed on infected animal,' i.e., it was a natural infection due to some agency outside the experiment.

In view of the ease with which these 'proboscis-and-gut' trypanosomes are propagated in nature by agents other than tsetse-flies, it is, in my opinion, unsafe to carry out experiments at a spot where the disease is already existent, especially if ruminants are employed to demonstrate the transmission.

In 1911, Fraser and Duke carried out seven transmission experiments with laboratory bred flies. One experiment was successful, trypanosomes appearing in the blood of the clean monkey on the eighty-fifth day after the first infecting feed of the flies. On the ninety-sixth day of the experiment a fly died which showed a heavy proboscis-infection. This fly, after infecting the clean monkey, had had access to two other clean monkeys, upon one of which it certainly fed several times. Neither of these last two animals became infected. At the time, it was supposed that the insect must have lost its infectivity, but I now believe that the explanation lay in the different resistance of the host-animals.

In the dissections of these experiments, twenty positive flies were found in a total of four hundred and twenty-seven dissected. Among these were five flies with flagellates established in the proboscis. One of these infected the monkey in the positive

experiment. Two occurred in another experiment, and fed repeatedly on a clean monkey without causing infection; they never had access to a ruminant animal. The remaining two flies with proboscis-infections occurred in a third experiment, and died on the fifty-ninth and seventy-fourth days after the original infecting feed; they both fed repeatedly on a clean monkey without ever infecting it.

The contents of the proboscis of all these five positive flies were injected into rats, without infection resulting. In these experiments no infection of the proboscis was met with in flies dissected before the fiftieth day after the first infecting feed. The salivary glands of these positive flies were all negative to flagellates. In all these experiments the clean animal employed was a monkey. No trypanosome disease existed in the vicinity of the laboratory at the time.

In 1911-12 experiments were carried out on the transmission of *T. nanum* by laboratory-bred *G. palpalis* in Uganda. In the first set of experiments a goat was used for the infecting feeds; one hundred and seventy-three flies were dissected during these experiments; no infected flies were obtained and no transmission occurred.

In the next series of experiments an infected sheep was employed, on which the flies fed much more readily, and the parasite was transmitted to a clean calf. Three hundred and twenty-two flies were dissected, of which twelve were infected, five showing flagellates established in the proboscis. In only one of the proboscis-infections were flagellates seen in the hypopharynx; the labrum of this fly contained great clusters, while a few individuals were present in the hypopharynx. This fly died on the twenty-fifth day after the first infecting feed—the earliest recorded date for the infection of the proboscis of *G. palpalis* by a member of this group of trypanosomes.

In 1911-12 another series of transmission experiments were carried out with *T. congolense* and laboratory-bred *G. palpalis*. The animal used for infecting the flies was a young bushbuck which had been born at the laboratory and, when a few months old, had been infected by syringe inoculation with the Mpumu Laboratory strain. In the course of these experiments, seven hundred and forty-six flies were dissected, of which six hundred and thirteen lived

until the thirtieth day after their first feed on infected blood. Fifteen flies were found to contain flagellates; four had proboscis-infections; and one a heavy infection of the sucking-stomach, with no flagellates in the proboscis. The box which was apparently responsible for the successful transmission, contained this fly with the infected sucking-stomach and none with infected proboscis. The four flies with infected proboscides died respectively on the seventy-sixth, one hundred and fourth, and one hundred and forty-first days after the first infecting feed. A ninetieth day fly showed flagellates in the hind-gut only. |

It must be noted that the strain of *T. congolense* kept at the Mpumu Laboratory was derived from cattle, from localities where tsetse-flies are absent or scarce. It is, therefore, probable that it was a directly transmitted strain, before it commenced its career at the laboratory. Too much stress, therefore, cannot be laid upon its behaviour when exposed to tsetses.

The strain of *T. nanum*, on the other hand, came from cattle in the *G. pallidipes* country in Toro Kingdom, and Sheep Experiment 59, by which the flies were infected, was the second passage animal from the original ox.

As already stated, the strain of *T. congolense* used in the experiments now to be set forth is known to be a cyclically carried wild fly strain.

### III. HISTORY OF THE TRYPANOSOME STRAIN EMPLOYED IN THE TRANSMISSION EXPERIMENTS PERFORMED AT ENTEBBE DURING 1922 and 1923

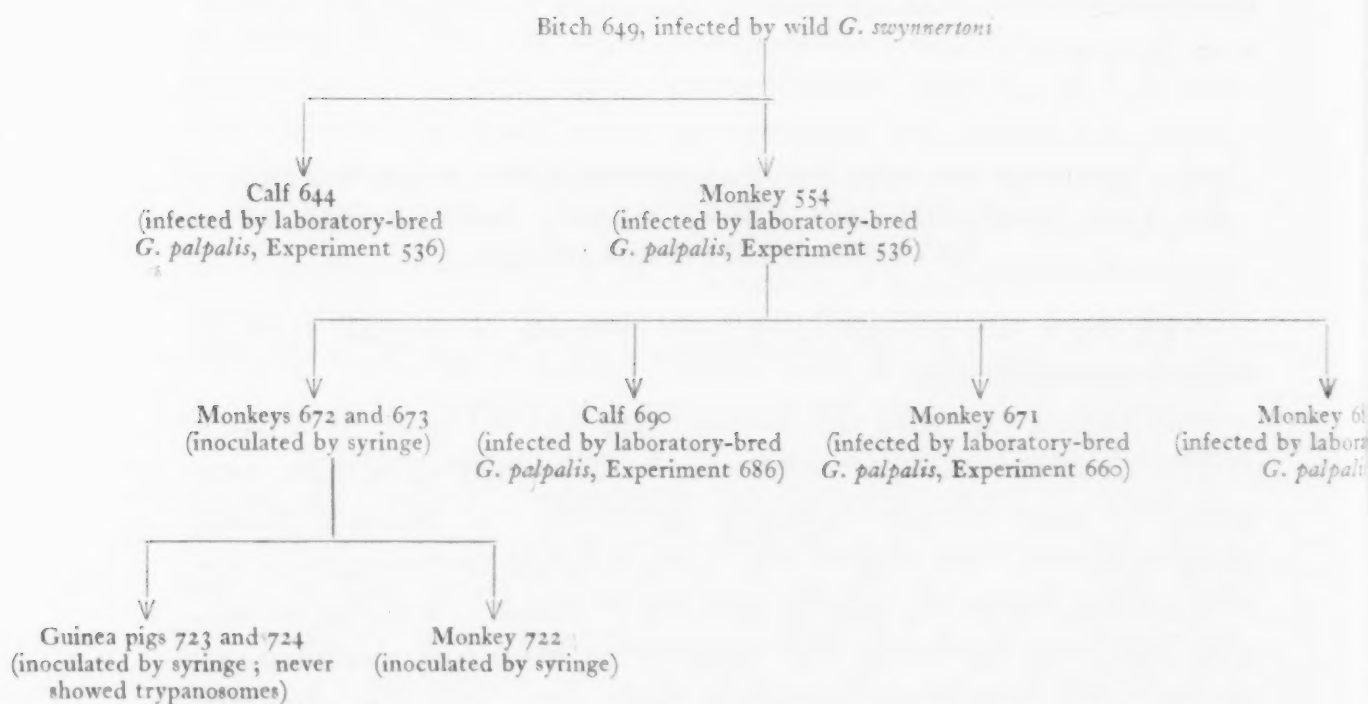
This strain was brought back from Mwanza, in August, 1922, in Bitch Experiment 649.

On August 1st, 1922, Experiment 649 was fed upon by a batch of wild *G. swynnertoni*, of which two, on subsequent dissection, were found to have proboscis-and-gut infections, the salivary glands being negative. On August 8th, 1922, a single trypanosome of the *T. congolense* type was seen in the animal's blood. On August 9th, 10th and 11th, the blood was negative to fresh film examination; on the 12th a few trypanosomes were seen, and thereafter the animal was negative to daily examination of stained thick films

until September 13th. From this date trypanosomes were found at frequent intervals in the course of the daily routine examinations, until February 2nd, 1923. Then followed a series of negative examinations until February 28th, when a few parasites were seen in a stained thick film. Trypanosomes were next seen, on a single occasion only, on August 20th, 1923. For a month or so after arriving at the Entebbe Laboratory, the animal was languid and seemed to be losing condition. In October she showed signs of being pregnant; the mammae enlarged and the animal became fatter, but in a week or two these symptoms disappeared. The general condition was now excellent. She became pregnant about the middle of June, and on August 24th, 1923, at 12 o'clock, two healthy puppies were born, followed, an hour and a half later, by a third which died an hour after birth.

The subsequent upkeep of the strain from this bitch is shown in Table I.

TABLE I.





#### IV. ANIMAL REACTIONS OF THE STRAIN OF *T. CONGOLENSIS* REFERRED TO IN THIS PAPER

The *T. congolense* isolated by Bruce's Commission and employed in their original transmission experiments at Mpumu, lost some of its virulence during two years of maintenance at that laboratory. The average duration of the disease in ten completed monkey experiments carried out by Bruce was sixty-three days; one animal died after one hundred and eighty-one days, the average for the other nine being forty-eight days. Another monkey was alive after two hundred and sixteen days. In eight completed dog experiments the average duration was forty-three days. Of three monkeys inoculated with the same strain eighteen months later, one died after thirty-five days, one after one hundred and fifty days, and the other was still alive after two hundred and three days.

The animal reactions of the strain from the Mwanza Fly Belt are as follows:—

TABLE II.

	Incubation period, in days					Duration of disease, in days
Monkeys—						
554 ...	...	78 days or more	...	...	127 at least.	
671 ...	...	47 days or more	...	..	Still alive after 145 days.	
689 ...	...	?			47th day after first appearance of trypanosomes of Fly Experiment 686.	
672 ...	...	16	...	...	Still alive after 188 days.	
673 ...	...	16	...	...	64 (trypanosomes swarming in blood).	
722 ...	...	38	...	...	43 (trypanosomes swarming in blood).	
Calves—						
640 ...	...	10	...	...	Alive and apparently well after 7 months.	
644 ...	...	8	...	...	Alive and apparently well after 9 months.	
690 ...	...	23-25	...	...	Alive and apparently well after 107 days.	



#### V. TECHNIQUE OBSERVED IN THE CONDUCT OF THESE TRANSMISSION EXPERIMENTS

The transmission experiments were carried out according to the methods pursued for many years in the Uganda Laboratory. The newly hatched flies are placed in wire-sided boxes and fed and starved on alternate days, dead flies being removed for dissection each morning. During the act of feeding, a wet rag covers the top side of the box, while the other side is closely applied to the animal's skin. Feeding continues until the flies have had all the blood they want. At the termination of the experiment the survivors are killed with chloroform vapour and dissected. Experience has proved that the rough and ready method of holding the box in the smoke of a fire, though effective in killing the flies, also kills the flagellates they contain, and makes the identification of light infections more difficult. Throughout the experiments the boxes are kept on stones resting in dishes of clean water.

Whenever a fly with a proboscis-infection was found, the animals upon which the insect had fed were examined daily by means of a stained thick blood-film. Otherwise, all experimental animals were examined daily by careful inspection of fresh unstained blood-films.

## VI THE TRANSMISSION EXPERIMENTS

The actual experiments will now be set forth. In the 'positive-flies' column the contents of the brackets refer to the number of flies with infected proboscides. In the 'remarks' column will be found the result of the experiment, positive or negative, according as the clean animals develop trypanosomes or not.

(A) *Experiments in which some of the flies developed proboscis-infections.*

(1) Experiments in which cyclical transmission of *T. congolense* from sick to healthy animal occurred.

TABLE III.  
EXPERIMENT 536.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1922 Sept. 25-27	1-3	Flies fed on bitch 649 ...	...	<i>T. congolense</i> not seen in stained thick films of bitch's blood.
" 28	4	Starved ... ..	...	
" 29-Nov. 21	5-58	Fed on clean monkey 554	2 (1 proboscis +)	<i>T. congolense</i> first seen in 554's blood on 13 Feb., 1923.
Nov. 22	59	Starved ... ..	...	<i>T. congolense</i> first seen in calf's blood on Dec. 1.
" 23-Dec. 1	60-68	Fed on clean calf 644 and on 554 on alternate days	...	
Dec. 2	69	Starved ... ..	...	Monkey 611 never showed trypanosomes.
" 3-4	70-71	Fed on clean monkey 611	...	
" 5	72	Remaining 28 flies dissected	3 (1 proboscis +)	

Flies dying before 16th day were ignored.  
Total number of flies dissected during the experiment = 68.  
Number alive on 25th day of the experiment = 64.

*Remarks.* November 27th was the last date on which the flies had access to 554, the incubation period in the monkey was thus very long, amounting to at least seventy-eight days. In contrast to this the incubation period in the calf was only nine days.

TABLE IV.  
EXPERIMENT 660.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923 Feb. 16	1	Flies fed on monkey 554	...	<i>T. congolense</i> present in 554's blood.
" 17	2	Starved ... ..	...	
" 18-19	3-4	Fed on monkey 554 ...	...	<i>T. congolense</i> present in 554's blood.
" 20	5	Starved ... ..	...	
" 21-23	6-8	Fed on monkey 554 ...	...	<i>T. congolense</i> present in 554's blood.
" 24	9	Starved ... ..	...	
" 25-April 8	10-52	Fed on clean monkey 671 on alternate days	1 (proboscis +++) 51st day	Monkey 671 first showed <i>T. congolense</i> on 24 May, 1923
April 9-10	53-54	Starved ... ..	...	
" 11	55	Remaining 19 flies dissected	2	

Flies dying before the 22nd day of the experiment were ignored.

Total number of flies dissected = 58.

Number alive on 25th day of experiment = 55.

*Remarks.* Note the long incubation period of *T. congolense* in monkey 671, i.e., from, say, the first week in April until 24th of May.

TABLE V.  
EXPERIMENT 686.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923 Mar. 11-13	1-3	Flies fed on monkey 554	...	<i>T. congolense</i> present in 554's blood.
" 14-15	4-5	Starved ... ..	...	
" 16-May 13	6-64	Fed on clean monkey ...	1 (proboscis +) 54th day	Monkey 689 first showed <i>T. congolense</i> in blood on 23 May, 1923.
May 14	65	Starved ... ..	...	
" 15-17	66-68	Fed on clean calf 690 ...	...	Calf 690 first showed <i>T. congolense</i> in blood on 7 June, 1923.
" 18-19	69-70	Starved ... ..	1 (proboscis +) 69th day	
" 20	71	Fed on 689 ... ..	...	
" 21	72	Starved ... ..	...	
" 22	73	Fed on 689 ... ..	...	
" 23-24	74-75	Starved ... ..	...	
" 25	76	Remaining 14 flies dissected	...	

Flies dying before 12th day were ignored.  
Total number of flies dissected = 49.  
Number alive at 25th day = 46.

*Remarks.* It is impossible to estimate the incubation period in monkey 689. In the calf, trypanosomes appeared twenty-three to twenty-five days after the infecting feed.

## (2) Experiments in which no transmission occurred.

TABLE VI.  
EXPERIMENTS 597-8.

Date	Day of Experiment	Procedure	Positive Flies found	Remarks
1922 Nov. 18	1	Flies fed on dog 649 ...	...	<i>T. congolense</i> not seen in dog blood.
" 19	2	Starved ... ..	...	
" 20-21	3-4	Fed on dog ... ..	...	<i>T. congolense</i> not seen in dog's blood.
" 22	5	Starved ... ..	...	
" 23-Dec. 25	6-38	Fed alternate days on clean monkey 602	...	Monkey 602 never showed trypanosomes: examined by thick stained films till 15 June, 1923.
Dec. 26	39	Starved ... ..	...	
" 27	40	Fed on clean dog X ...	...	Dog X never showed trypanosomes.
" 28	41	Starved ... ..	...	
" 29	42	Fed on dog X ... ..	...	
" 30	43	Starved ... ..	2 (1 proboscis +)	This fly had fed on dog X.
1923 " 31-Jan. 16	44-60	Fed on alternate days on monkey 602 and dog X	...	
Jan. 17	61	Remaining 32 flies dissected	...	

Flies dying before the 11th day of the experiment were ignored.  
Total number of flies dissected = 113.  
Number alive at 25th day of experiment = 105.

*Remarks.* Neither of the clean animals of this experiment became infected. The infection of the proboscis in the forty-three day fly was not heavy, and may have been established after the fly had been removed from contact with monkey 602.

Possibly, in the earlier stages of the invasion of the proboscis, the fly is only capable of infecting very susceptible mammals, on account of the small number of trypanosomes which it inoculates.



TABLE VII.  
EXPERIMENT 658.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923 Feb. 15-16	1-2	Fed on monkey 554 ...	...	<i>T. congolense</i> present in 554's blood.
" 17	3	Starved ... ..	...	
" 18	4	Fed on monkey 554 ...	...	<i>T. congolense</i> present in 554's blood.
" 19	5	Starved ... ..	...	
" 20	6	Fed on monkey 554 ...	...	<i>T. congolense</i> present in 554's blood.
" 21	7	Starved ... ..	...	
" 22	8	Fed on monkey 554 ...	...	<i>T. congolense</i> present in 554's blood.
" 23	9	Starved ... ..	...	
" 24-April 21	10-66	Fed on clean monkey 668	1 (proboscis +) 66th day	Monkey never showed trypanosomes: examined daily, stained thick films till 23 July, 1923.
April 22	67	Starved ... ..	...	
" 23-25	68-70	Dog T' ... ..	...	Dog never showed trypanosomes.
" 26	71	Starved ... ..	...	
" 27-May 1	72-76	Fed on monkey 668 and starved alternate days	...	
May 2-3	77-78	Fed on clean calf ...	...	Calf never showed trypanosomes.
" 4-6	79-81	Starved ... ..	...	
" 7	82	Remaining 12 flies dissected	...	

Flies dying before the 17th day were ignored.  
Total number of flies dissected = 41.  
Number alive on 25th day = 47.

*Remarks.* The only positive fly of this experiment fed repeatedly on monkey 668 without causing infection. Unfortunately this fly died before feeding on the dog or the calf.

(B) *Experiments in which no flies with infected proboscides were found.*

(In none of these experiments did transmission occur.)

Table VIII gives a summary of these experiments.

TABLE VIII.

Experiment	Date of infecting feeds	Infecting animals	NUMBER OF FLIES			Duration of Experiment in days	Day of Experiment on which dissection began
			Alive 25th day	Dissected during Experiment	Containing flagellates in gut		
520	19-21.9.22 ...	Bitch 649 ...	59	63	0	77	18th
600-1	22-24.11.22 ...	Bitch 649 ...	85	123	0	57	13th
643	9, 10 and 12.2.23	Calf 644 ...	58	66	0	44	16th
662	18-21.2.23 ...	Calf 644 ...	40	54	2	68	13th
665	21-23.2.23 ...	Calf 644 ...	37	37	0	65	24th
664	19-21 and 23.2.23	Monkey 544 ...	46	50	0	77	18th
667	23-25.2.23 ...	Calf 644 ...	53	63	0	61	11th
674	1-3.3.23 ...	Calf 644 ...	39	46	0	74	15th
678	3-6.3.23 ...	Calf 644 ...	46	47	0	72	19th
684	8-10.3.23 ...	Monkey 554 ...	29	31	0	78	16th
693	15-17.3.23 ...	Monkey 673 ...	50	53	1	83	19th
700	19-22.3.23 ...	Monkey 554 ...	44	53	0	82	14th
			586	686	3		

The results of dissection of the positive flies of all the above experiments are shown in Table IX.

TABLE IX.

Experiment	Number of fly	Day of dissection reckoned from first infection feed	Distribution of flagellates		Remarks
			Gut	Proboscis	
693	1	20th day	++	o	
662	2	21st day	+++	o	
536	3	26th day	+++	o	
543	4	34th day	+++	o	
579-8	5	43rd day	+++	o	
...	6	43rd day	+++	+	
660	7	51st day	+++	+++	
543	8	52nd day	+++	+++	
662	9	53rd day	+++	o	
686	10	54th day	+++	++	
660	11	55th day	+++	o	
...	12	55th day	+++	o	
536	13	57th day	+++	+++	Sucking stomach +++
642	14	58th day	+++	o	
536	15	62nd day	+++	o	
658	16	66th day	+++	+++	
686	17	69th day	+++	++	
536	18	72nd day	+++	o	
...	19	72nd day	+++	+++	
...	20	72nd day	+++	+++	

## VII. DISCUSSION OF THE EXPERIMENTS

It will be seen from Table IX that the earliest date at which flagellates were found in the proboscis was in a forty-three day fly. It is, however, possible that the invasion of the proboscis in some of the older flies took place earlier than this. On the other hand,

several infected flies lived considerably longer than forty-three days without the flagellates reaching the 'anterior station.'

The experiments of Group A show that flies with heavily infected proboscides may feed upon a clean animal without causing infection. It is interesting to note that, when this happened, in each case the clean animal was either a monkey or a dog: whenever a calf was bitten by a fly with an infected proboscis, the animal developed trypanosomes.

It was hoped, by exposing, alternately, different species of clean animals to the infected flies of these experiments, to throw light upon the biological significance of the differences in virulence and of host proclivities shown by the trypanosomes of this group. But, unfortunately, on several occasions the infected fly died before the box was transferred to a second, or a third clean animal. It was thus not possible to ascertain whether a fly may be infective to a ruminant and yet be unable cyclically to infect a monkey or a dog.

The strain of *T. congolense* used in these experiments was of comparatively low virulence. On several occasions the incubation period in monkeys, before trypanosomes appeared in the peripheral blood, was very long. In the case of monkeys Nos. 554 and 671, both of which were infected by flies, the incubation periods were seventy-eight and forty-seven days, respectively; with monkey Experiment 722, infected by the syringe, thirty-seven days elapsed before trypanosomes were detected. The incubation periods in the calves infected by flies were nine and twenty-three to twenty-five days. Similarly, in my experiments at Mpumu, the incubation period in the only monkey infected by cyclical transmission was thirty-three days, while in another monkey, infected by means of the syringe, the incubation period was forty-seven days.

In contrast to this, the average incubation period in thirteen monkeys inoculated by Bruce (1910) in Uganda was 12·3 days, maximum twenty-one days. Bruce was employing a virulent strain—probably directly transmitted—derived from a cattle epidemic. This strain was maintained for upwards of two years by syringe inoculation from monkey to monkey before being subjected to the transmission-experiments just referred to.

Both the *nanum* and the *congolense*-forms of this group—distinguished from one another by their different behaviour in

monkeys and dogs—have been recovered from wild game tsetses, the *nanum*-form being the most common. The *congolense*-form is acknowledged to be the more virulent, and is almost always present in cattle epidemics due to this group of trypanosomes. The evidence supplied by the experiments set forth in Section 6, taken in conjunction with the observations already recorded on this group of trypanosomes, suggests that cyclical transmission of *T. congolense* tends to the acquisition by the trypanosome of a low degree of virulence, which may be associated with inability to infect such animals as monkeys and dogs in nature. Directly transmitted natural strains, on the other hand, usually possess greater virulence, and readily infect these two animals.

#### SUMMARY

1. Three out of seventeen cyclical transmission experiments, performed with laboratory-bred *G. palpalis* and a wild fly strain of *T. congolense*, were successful. In the course of these seventeen experiments, one thousand and fifteen flies were dissected, of which eight hundred and ninety-three lived until the twenty-fifth day after their first feed on an infected animal; eight of these flies had flagellates established in the proboscis.

2. In all the successful transmission experiments, one or more flies with proboscis-and-gut infections had fed upon the clean animals which acquired infection. There is every reason to believe that these flies were responsible for the transmission.

3. Flies with equally intense proboscis-and-gut infections were found in two experiments in which no transmission occurred. Several of these flies had fed repeatedly on clean monkeys without infecting the animals; in no case, however, did a fly with a proboscis-and-gut infection feed upon a clean calf without infecting it. It would appear, therefore, that monkeys are less susceptible to this strain of *T. congolense*, carried by *G. palpalis*, than are calves.

4. In several cases the incubation period in the monkey was very prolonged.

5. The strain of *T. congolense* used in the experiments is less virulent than the strain used by Bruce at Mpumu, in Uganda.



The Mpumu strain was almost certainly directly transmitted before its arrival at the laboratory, while the strain here described was carried cyclically by wild tsetse. It is possible that there is a definite relation between the virulence of a strain and its method of transmission.

6. The apparent fact that the wild *G. palpalis* of the Uganda shores of Victoria Nyanza do not carry trypanosomes of the proboscis-and-gut group is to be explained, in part at any rate, by the partiality of the fly for animals which are not susceptible to this group of trypanosomes. It would appear, however, from the experiments above described, that this *G. palpalis* is less fitted to act as a true intermediate host of the *T. congolense* group of trypanosomes than of *T. vivax*, *T. uniforme*, and *T. brucei*.

My thanks are due to Dr. Mary Martin, Assistant Bacteriologist, Uganda Protectorate, for valuable help in the conduct of these experiments.

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## ON A NEW SPECIES OF *PHLEBOTOMUS* FROM JAPAN

BY

R. NEWSTEAD, F.R.S.

*(Received for publication 15 October, 1923)**Phlebotomus squamirostris*, n. sp.

*Superior claspers of the male with two pairs of stout spathuliform spines, the proximal pair arising from a well-developed tubercle. Terminal segment of the palpi slightly longer than the fourth. Rostrum (labium) densely scaly.*

*Male.* Abdominal hairs recumbent and uniformly pale ochraceous as on other parts of the body. Wings faintly iridescent; costal hairs scarcely darker than the rest. Rostrum (labium) (fig. 1 *e*) densely clothed with long, forwardly directed, non-deciduous scales. Antennae with relatively long segments, and short, unilateral, geniculated spines; third segment (fig. 1 *a*) projecting beyond the tip of the proboscis to a distance of 0.1 mm. or more, the geniculated spine placed near the distal fourth. The spine on the other segments is placed proximally a little in advance of the articulation (fig. 1 *b*). Palpi rather robust; the third segment distinctly incrassate, the fifth about one-third longer than the fourth; formula 1 (2, 4), 3, 5. Wings (fig. 1 *c*) lanceolate; the anterior branch of the second long vein about equal in length to the distance between the forks. Armature (fig. 1 *d*); superior claspers with two pairs of stout spathuliform spines, arranged in two pairs; the first attached to a well-marked tubercle slightly beyond the middle of the segment; the second pair terminal, each arising from a tubercle. Inferior claspers relatively short, and either equal to, or very slightly longer than the proximal segment of the superior claspers.

Length: 2.7 mm.; length of wing: 1.6 mm.; antenna: 2.3 mm.

*Female.* Arrangement of abdominal hairs and colour as in the male; but the body is slightly more robust.

JAPAN:—Agori: July 19th, 1916, 1 ♂ (*Dr. Shinichiro Yamada*);  
Matzuyama: June 25th, 1916, 1 ♂, 1 ♀ (*Dr. S. Komatsu*).

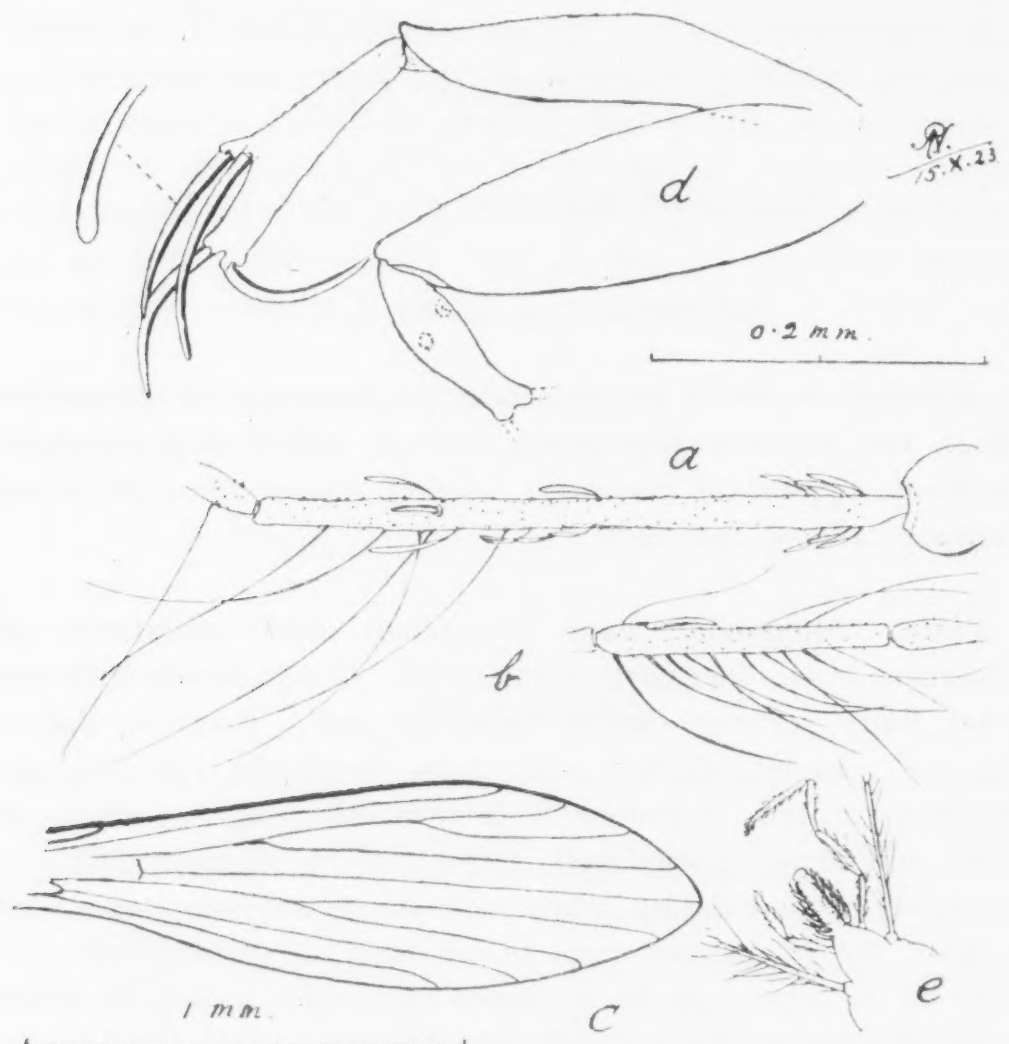


FIG. 1. *Pblebotomus squamirostris*, Newstead. ♂. *a, b*—Antenna; *c*—Wing; *d*—Armature; *e*—Rostrum. *a, b* and *d* to same magnification.

I am indebted to Dr. S. Yamada, Institute of Infectious Diseases, Imperial University of Tokyo, Japan, for the opportunity of studying this material.

*GLOSSINA ZIEMANNI*, GRÜNBERG, A  
SYNONYM OF *GLOSSINA PALPALIS*  
SUB-SPECIES *FUSCIPES*, NEWSTEAD

BY

R. NEWSTEAD, F.R.S.

(Received for publication 15 October, 1923)

In April, 1912, Dr. K. Grünberg\* described the tsetse-fly, *Glossina ziemanni*, which he considered distinguishable from all other known species by its uniformly dark colouration, its heavily infuscated wings and its entirely black tarsi. Furthermore, by the metallic sheen or iridescence of the scutellum and parts of the abdomen; and by the presence also of a dirty ashen grey pollinose covering. His description was based upon 1 ♂ and 5 ♀♀ (preserved in alcohol), which were taken at Mina, Mbam R., Cameroons, in 1912, and forwarded to Berlin by Dr. H. Ziemann.

Being desirous of examining examples of the species in question, I applied to Dr. G. Enderlein, of the Berlin Zoological Museum, for the loan of specimens of both sexes; this he very willingly granted, and at the same time forwarded the type ♂ and ♀ to this School.

On making an examination of the external characters, one saw at once that the remarkable iridescent colouration, the 'pollinose' covering and the deep blackish brown tinge of the wings were clearly caused by impurities in the alcoholic preservative; these impurities had so completely masked the true colours and pattern that it was quite impossible to determine the species without making a microscopical examination of the armature.

Having dissected out the structures—a task of great difficulty owing to the intense hardening of the integument—one is now able to say, quite definitely, that the morphological characters are specifically identical with those of *Glossina palpalis* sub-species *fuscipes*, Newst.

\* Eine neue Tsetse-fliege aus Kamerun: *Sitzgsber. Ges. Naturf. zu Berlin*. Jahrg. 1912, No. 4, p. 246 (April, 1912).

One may add that the presence of foreign matter on the wings is easily demonstrable by passing a beam of light through the membrane, when almost the entire surface can be seen to be more or less covered by dark granular bodies which, in places, have blotted out the true character of the membrane and have also matted the fine hairs together in many parts.

One regrets exceedingly to have to relegate Dr. Grünberg's species to the synonymy of *Glossina palpalis* sub-species *fuscipes*, Newst., but the study of the taxonomic characters of his material affords convincing proof that *G. ziemanni* must sink.

We tender our sincere thanks to Dr. Enderlein for his great kindness and courteous assistance, without which it would have been quite impossible to clear up the synonymy.



# MALIGNANT GROWTHS IN NATIVES OF SIERRA LEONE

BY

S. ADLER

AND

E. H. TAYLOR CUMMINGS

*(From the Sir Alfred Lewis Jones Research Laboratory)*

*(Received for publication 1 November, 1923)*

It is impossible to form an opinion on the frequency of malignant growths in natives of West Africa, as the aborigines seldom consult medical men. Nevertheless, it has often been stated that malignant growths are rare or absent in West Africans, and this has been attributed to non-adoption of European habits. As a proof of the alleged relationship between civilised habits and malignant growths, it has been stated that such growths have only been recorded among Creoles who have more or less adopted European habits, and in those living in coastal towns where European influence is active. Thus Renner (1910) states that cancerous and other malignant growths have been increasing among the Creoles of Sierra Leone in recent years, but that they are rare or absent in aborigines. He further states, that although the Fantis of the Gold Coast have been in contact with Europeans for centuries, malignant growths are rare or absent among them, because they have resisted the inroads of European civilisation. Macfie (1922), on the other hand, writing of the prevalent diseases of the Gold Coast, states:—‘Tumours are probably as common as elsewhere, but sarcomas appear to be rather commoner and carcinomas are said to be rare, a belief which may be due to the fact that the hospital clientèle represents only a small and selected portion of the total sick.’ Dyce Sharpe (1923) states that carcinomas are rare, differing in this respect from sarcomas, even among the population of the coastal towns of West Africa. Cameron Blair (1923) states that he has never seen a case of carcinoma or

sarcoma in twenty-two years in Nigeria, and that the occasional carcinomas found by medical men in the coastal regions, occur chiefly in natives who have come in contact with Europeans. It must be borne in mind, however, that Europeanisation, whether or not it were conducive to the spread of malignant growths, would certainly be responsible for intelligent natives afflicted with them consulting medical officers. Thus civilisation may be wrongly blamed for the spread of the disease, when it is only responsible for its diagnosis.

In view of the alleged rarity of malignant growths in West African natives, the following record of seven cases, five of which came under our personal observation between May and November, 1922, may be of interest. Five of these cases occurred in aborigines and two in Creoles.

CASE 1. An aborigine (male Timne, aged 40 circ.) gave a history of an ulcer on the plantar surface of the right foot following an injury. When seen by us there was a fungating growth from the base of a chronic ulcer, which on section proved to be a melanotic sarcoma. There was a large secondary growth in the right groin.

CASE 2. An aborigine (male Timne, aged 60 circ.) had a painful growth on the scrotum which was found on section to be an epithelioma.

CASE 3. A Creole (male, aged 58) had an ulcer which commenced on the upper lip. Sections showed the ulcer to be an epithelioma. There were secondaries in the glands of the neck on both sides.

CASE 4. An aborigine (male Timne, aged 50 circ.) had a tumour on the right side in the temporal region, exophthalmos of the right eye and complete hemiplegia on the left side. *Post-mortem*: Meningo-sarcoma which had destroyed a large area of bone on the right side, involving the temporal, frontal parietal and sphenoidal bones, infiltrated the muscles and subcutaneous tissue, and also invaded the right orbit. There were large secondaries in the liver.

CASE 5. An aborigine (male Mandingo, aged 28 circ.) died in the Colonial Hospital, Freetown. Dr. J. D. Dimock, W.A.M.S., found a tumour in the liver, which he kindly presented to the Sir A. L. Jones Research Laboratory. Sections of the tumour showed it to be primary carcinoma of the liver.

CASE 6. A Creole (female, aged 42) complained of debility. *Post-mortem*: Tumour of the liver, which on section proved to be a primary carcinoma.

CASE 7. Dr. C. H. Allan, W.A.M.S., sent a piece of liver containing growth which he obtained from a post-mortem on an aborigine (Sherbro). Sections showed the liver to be invaded by a secondary carcinoma.

In addition to the above material, we examined a tumour of the breast which Capt. M. Jackson, W.A.M.S., removed from a Mende woman, aged 50 (circ.). The tumour on section proved to be a carcinoma.

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# A PRELIMINARY ACCOUNT OF THE RESULTS OF SURVEYS FOR BREEDING- PLACES OF MOSQUITOES IN NORTH WALES

BY

W. REES WRIGHT, B.Sc.

*(Received for publication 5 November, 1923)*

## INTRODUCTION

The present paper is an account of the results of an investigation into the biology of the species of CULICIDAE, or mosquitoes, present in various parts of North Wales. The work, which was carried out under the Department of Zoology and the Agricultural Zoology Laboratory of the Department of Agriculture, at University College, Bangor, was begun in October, 1920, and has since been prosecuted whenever circumstances have permitted; that portion of it chiefly dealt with here, viz., observations on breeding-habits, was carried out during the summers of 1921, 1922, and 1923.

In the summer of 1921, my attention was entirely confined to an area of CARNARVONSHIRE bounded by the MENAI STRAITS, the OGWEN RIVER, the SNOWDONIAN MOUNTAINS, and the GWYRFAI RIVER. The elevation of the area worked varies from a few feet above sea-level to about 480 feet at LAKE CWELLYN. The major part of the district is well above the 200-feet contour; the only low-lying land is along the shore of FORYD BAY, an arm of the Menai Straits. In this part there are a relatively large number of slow running ditches and brackish pools. In the rest of the area there are few ponds; drainage is largely by swiftly running natural streams.

The fields are for the most part bounded by stone walls without ditches. The area is moderately wooded; the preponderating trees are oaks and conifers; such tree-hole examination as has been done has yielded negative results (Blacklock and Carter (1920)).



In the summer of 1922, and again in 1923, I re-surveyed the North Carnarvonshire area, while I examined, in addition, streams and ground collections of water in the neighbourhoods of DOLGELLEY and DINAS MAWDDWY, MERIONETHSHIRE, and ABERFFRAW, ANGLESEY (this last in 1922 only).

In all, ten species of mosquitoes have been taken in the larval stage; viz., *Anopheles maculipennis*, *A. bifurcatus*, *Aedes* (*Ochlerotatus*) *detritus*, *A. (O.) caspius*, *A. (O.) punctor* var. *meigenanus*, *A. (O.) rusticus*, *Aedes* (*Ecculex*) *vexans*, *Theobaldia annulata*, *Theobaldia (Culicella) morsitans*, and *Culex pipiens*. The first three and last four have also been obtained as imagines. The nomenclature employed is that of F. W. Edwards (1921); since this differs from that employed previously (*e.g.*, Lang (1920)), I have thought it advisable to give both the authority for the name, and also, when Lang's specific or generic name differs from that of Edwards, that employed by the former author.

It may be mentioned that the weather varied during the period which this paper covers, from being very dry and warm (1921) to cold and wet (1923).

I have very great pleasure in taking this opportunity of acknowledging my indebtedness to Professor P. J. White, F.R.S.E., Department of Zoology, and Mr. C. L. Walton, Adviser in Agricultural Zoology at Bangor, for much valuable advice, assistance and encouragement; to Professor Warrington Yorke, Professor R. Newstead, F.R.S., and Miss A. M. Evans, of the Liverpool School of Tropical Medicine for valuable assistance in the matter of literature; to Professor Newstead, for helpful criticism also; to Mr. F. W. Edwards, of the British Museum (Natural History), for information as to literature, and for the identification of imagines of *Theobaldia morsitans*; and finally to numerous landowners and farmers, especially the Trustees of the Vaynol Estate and Mr. W. H. Jones, Plas Llanfaglan, Carnarvon, for affording me every facility for the 'field-work' involved in these investigations.

*Anopheles maculipennis*, Meig.

*Anopheles maculipennis*, Meigen. Syst. Besch., Vol I, p. 11 (1818).

This species is everywhere common. In my experience, it will

breed in any moderately clean water containing vegetation. The presence of a moderate amount of current does not affect it; it will breed in swiftly running streams, provided there is enough vegetation to shield the larvae from the full force of the current. As for volume of water, it needs but little. In the laboratory, the larvae may be bred in shallow vessels, offering a large surface to the air, with low mortality; when the surface is small relative to the volume, there is considerable mortality, even if the water be daily artificially aerated.

According to Martini (1921), the Sanitary Staff of the German Army in Macedonia found that this species would not breed in water thickly covered with duckweed. I have obtained larvae from pools thickly covered with *Lemna minor*. I have also obtained larvae from water 'choked' with *Elodea canadense*.

It is a well-known fact that in the later years of last century, and the earlier years of this, there was a continued decrease in the amount of endemic malaria ('ague') in Great Britain and also in other parts of Northern Europe (Wesenburg-Lund, 1921). Various theories have been put forward to account for this, the latest being that independently proposed by Wesenburg-Lund and Roubaud, that a change has taken place in the mode of nutrition of *Anopheles maculipennis*. It has been suggested by Nuttall (Lang, 1918) that unfavourable breeding seasons, by temporarily exterminating *Anopheles* spp. in the ague zones, may have broken the 'chain of infection,' and thus brought about the observed disappearance of the disease; he suggests that very wet seasons may bring this extermination about by washing-out larvae from the localities in which they normally breed. I doubt if this cause would operate in such country as is found in North Carnarvonshire, where there are few ditches, the majority of the Anopheline breeding in isolated pools, etc., not liable to 'wash-outs.' Ague was formerly endemic in parts of the North Carnarvonshire littoral; I have met several farmers who remember a time when it was often difficult to conduct agricultural operations owing to ague among the farm labourers. At the present time, there are thousands of Anophelines in the area, and many imported cases of malaria, but so far as I am aware, there have been no locally contracted cases of malaria within the last few years.

The observations of Macgregor (1921) and James (1922) in

Surrey, and my own in North Carnarvonshire, suggest that a dry season is not likely greatly to affect the Anopheline mosquitoes.

This species generally breeds in the least shaded situations, so that in pools or streams it may be expected on the North side, unless this be shaded, deep, or without vegetation. I have occasionally obtained it from quite shady spots.

On 13th September, 1922, I obtained larvae of this species from a rainwater tank at PLAS LLANFAGLAN, near Carnarvon.

Larvae in all instar, and pupae, may be obtained throughout the summer.

The females hibernate in the warmer farm buildings.

*Anopheles bifurcatus* (Linn.).

*Culex bifurcatus*, Linnaeus. Syst. Nat., Ed. X, p. 603 (1758).

This species is the commonest mosquito in North Carnarvonshire. It breeds in the same types of water as does *A. maculipennis*, and also in extremely foul pools in marshes. I have often obtained larvae of the two species together. This species may be bred in the laboratory under the same conditions as *A. maculipennis*.

Feytaud and Gendre (1919) state that, while *Anopheles maculipennis* 'develops above all in stagnant water, clean and sunny (clear pools, lagunes, marshes, etc.); with abundant vegetation, variable temperature,' etc., '*Anopheles bifurcatus* likes pure water . . . cold, with little vegetation. We especially see it in fresh springs, streams through woods . . . wells.' The results obtained by Boyd (1922) and myself are not in agreement with this; Boyd remarks that 'the dyke in which the greatest number (of larvae) were found has the banks overgrown with weeds, and is almost stagnant in parts. The bottom is covered with leaves and decaying vegetation, into which the larvae appear to burrow at times.'

Larvae may be found throughout the year in all instar, and pupae throughout the summer.

The larvae of both species of Anophelines commonly bear a greater or less number of ectozoic Ciliates, very similar in appearance to *Vorticella*. These occur but rarely on Culicine larvae.

The larva hibernates.

*Theobaldia annulata* (Schrank).

*Culex annulatus*, Schrank. Beit. Z. Naturg., p. 97 (1776).

This, the largest and most ornamented of our mosquitoes, is common everywhere.

I have never found the larvae except in clean currentless water, but it has elsewhere been found breeding in foul rainwater (R. Newstead). It may be found in both natural and domestic collections.

Normally, this species hibernates as the adult female, in cellars and lofts.

Larvae of this species hibernated, in the winter of 1921-22, in a tank at UNIVERSITY COLLEGE, BANGOR, attaining the adult condition at the end of March, 1922. During the winter the water in the tank was once frozen almost—if not quite—solid, while it was frozen on the surface only, on two other occasions. Boyd (1922) has made similar observations.

Wesenburg-Lund (1921) states that in Denmark this species is exclusively domestic.

*Theobaldia (Culicella) morsitans* (Theo.).

*Culex morsitans*, Theobald. Mon. Cul., Vol. II, p. 5 (1901).

*Culicella morsitans* (Theo.). Lang. Handbook, p. 102 (1920).

I have obtained this species from one locality only, in a wood near GLAN-RHYD FARM, PENTIR, near Carnarvon (altitude slightly over 350 feet), where I found numerous first and second instar larvae in pools in a ditch, partly filled with fallen leaves and similar debris, on 21st September, 1922. I visited this pool on several later occasions. During the winter, fourth instar larvae were found; these pupated during May and June, 1923. Imagines bred in the laboratory from larvae were sent to Mr. F. W. Edwards, and identified as this species. After May, no larvae of this species were found until 11th October, when I obtained a few. The ditch was full of water most of the time.

According to Wesenburg-Lund (1921), the eggs are deposited on dry earth during July and August. These hatch out in September, the larvae proceeding to the fourth instar during the period 1st October to 1st December. The winter is passed as fourth instar larvae.



*Aedes (Ochlerotatus) detritus* (Hal.).

*Culex detritus*, Haliday. Entom. Mag., Vol. I, p. 151 (1833).

*Ochlerotatus detritus* (Hal.). Lang. Handbook, p. 89 (1920).

This species has been obtained from the low-lying part of the Parish of LLANFAGLAN, bordering on FORYD BAY, and also from similar land near ABER, Bangor, in late August, 1923.

The larvae occur both among vegetation and in open water; in pools the large fourth instar larvae may often be seen in the clear water towards the centre. Usually it is found in brackish or salty water, but I have taken it from inappreciably saline water and also from slowly running fresh water. In the laboratory, the larvae are easy to breed; they can be kept in shallow dishes filled with water from their original breeding-place, fresh water being occasionally added to compensate for evaporation. I have kept larvae in two cubic centimetres of water apiece, with no losses. By gradually diluting the brackish water, the larvae may become accustomed to, and thrive in, fresh water.

Under laboratory conditions, larvae pupated at various times between 11 a.m. and 6 p.m. on 12th July, 1922, the first imago emerging about 4 p.m. on 16th July. The maximum temperature in the interval was 66° F., the minimum 58° F.

On 29th June, 1923, I placed some dry mud from a ditch in which this species had bred the previous year, in a large dish of water. On 5th July, I noticed a larva swimming in the dish. This cast its skin on the following day, and pupated on the 11th. On the 14th, a female emerged from the pupa. The mean temperature was slightly over 60° F.

The imagines spend the day in the vegetation, around the breeding-places; they may often be beaten out in large numbers. The females are vicious biters, both in nature and under laboratory conditions; the swelling after the bite is, in my experience, more painful than that of any other of our North Wales species. In the laboratory, the females will attempt to feed shortly after emerging from the pupae, before the chitin of the mouth-parts has become rigid enough to allow of piercing the skin.

According to Lang (1920), there are 'at least two generations in the year.' In 1922, I believe there were three in this area. In



late June and early July, and again in late August and early September, larvae were abundant; in the interim there were none. The major part of the second brood had attained the last larval instar at least by 13th September, 1922, when I found only fourth instar larvae and pupae. On 25th November, 1922, I visited several of the pools where I had found larvae during the summer, with the object of obtaining data regarding the hibernation of this species. All pools and ditches were covered with ice; on breaking this and dipping near the margin, I everywhere obtained numerous larvae. All four instar were taken, the first two predominating. This suggests that in the interval between my two visits (13th September and 25th November) the females of the second brood—*i.e.*, females hatched from the larvae and pupae of August-September—had oviposited and that from the eggs emerged the larvae found on 25th November, these being a third generation. Larvae in all instar continued to abound until June, 1923, when they disappeared. In July the pools and ditches dried up. In August they again filled, and larvae were found until 2nd October, 1923. On 31st October no larvae could be found anywhere after a careful search. James (1922) records that larvae were found throughout the year.

According to Wesenburg-Lund (1921) and Lang (1920), this species hibernates as the egg; apparently it can hibernate also as the larva. It survives drought as the egg.

*Aedes (Ochlerotatus) punctor* (Kirby) var. *meigenanus*, Dyar.

*Culex punctor*, Kirby. Fauna Boreali-Amer., Zool. Ins., p. 305 (1829).

*Aedes meigenanus*, Dyar. *Insecutor Inscitiae Mens.*, Vol. IX, p. 72 (1921).

*Ochlerotatus nemorosus* (Theobald). Lang. Handbook, p. 91 (1920).

I have obtained larvae of this species from pools in a marsh near LLANELLYD BRIDGE, DOLGELLEY (altitude well under 50 feet), in company with larvae of *Culex pipiens*, 27th July, 1922; and also nearer DOLGELLEY in company with *Aedes vexans*, 27th July, 1923.

*Aedes (Ochlerotatus) caspius* (Pallas).

*Culex caspius*, Pallas. Reise versch. Prov. Russ. Reich, Vol. I, p. 475 (1771).

*Ochlerotatus caspius* (Pallas). Lang. Handbook, p. 81 (1920).

I obtained larvae of this species in a pool on FAIRBOURNE (South of Barmouth, Merionethshire) Golf Links, 25th July, 1922; and with *Aedes vexans*, near DOLGELLEY, 27th July, 1923. This species has previously been recorded from Merionethshire (Tal-y-bont, North of Barmouth, see Lang, 1920) by Mr. F. W. Edwards.

*Aedes (Ochlerotatus) rusticus* (Rossi).

*Culex rusticus*, Rossi. Fauna Etrusca, Vol. II, p. 333 (1790).

*Ochlerotatus rusticus* (Rossi). Lang Handbook, p. 94 (1920).

Near DOLGELLEY, with *Aedes vexans*, 27th July, 1923.

*Aedes (Ecculex) vexans* (Meig.).

*Culex vexans*, Meigen. Syst. Besch., Vol. VI, p. 241 (1830).

*Ochlerotatus vexans* (Meig.). Lang Handbook, p. 85 (1920).

This species has been obtained from one locality; in a field on the right hand of the River Wnion, about half a mile below DOLGELLEY (altitude under 50 feet).

On the evening of 22nd July, 1922, I captured a number of imagines while beating a patch of rushes. On 24th July, a careful search led to the discovery of the larvae, in a small ditch.

In July, 1923, I again visited DOLGELLEY, and found this pool dry. On Monday, 22nd July, the pool was filled as a result of a flood. On Friday, 27th July, I obtained a large number of larvae. An attempt to breed the imagines failed, all the larvae dying, though one pupated. Most of the skins cast were preserved and mounted; a careful examination of this material showed it to contain, besides *Aedes vexans*—in all instar save the first—*Aedes caspius*, *Aedes punctor meigenanus*, *Aedes rusticus*, *Culex pipiens*, and *Theobaldia annulata*.

The imagines, apparently, spend the day among the vegetation. The females attack man.

Apparently, since larvae appear soon after the pool fills with water, it hibernates as an egg; no larvae could be found in the late autumn of 1922.

*Culex pipiens*, Linn.

*Culex pipiens*, Linnaeus. Syst. Nat., Ed. X, p. 602 (1758).

This species is everywhere common. It breeds, in my experience, in any type of water, natural or domestic. I have obtained the larvae from streams, ponds, pools in marshes, water in hoof marks, and all sorts of rainwater receptacles. It will breed in very foul situations. It is easy to breed in the laboratory.

The female hibernates in dark, cool cellars or lofts.

It rarely, if ever, attacks man. I have never succeeded in getting it to bite under experimental conditions.

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# THE FREQUENCY OF INDICANURIA

BY

R. M. GORDON

*(Received for publication November 6, 1923)*

## TEST EMPLOYED

To five ccs. of urine in a test tube were added one large drop of five per cent. potassium chlorate, then five ccs. of strong hydrochloric acid, followed by five ccs. of chloroform, the contents being mixed by inverting the closed test tube a couple of times. If no definite blue colouration appeared in the separated chloroform after thirty minutes, the result was regarded as negative.

## RESULTS OBTAINED

Three hundred and eighty cases were examined on one occasion each; of these, ninety were apparently healthy, normal individuals, twenty-two (24 per cent.) of whom gave positive results and sixty-eight (76 per cent.) negative. It is to be noted in this connection, that the same individual may give different results on various dates, or even at different hours on the same day. Thus a normal case tested on twenty occasions, was negative on twelve and positive on eight, a negative result frequently alternating with a positive. The remaining two hundred and ninety cases were undergoing treatment for various disorders. The results of the tests are shown in the table on page 550.

## REMARKS

The presence of indicanuria is usually attributed to excessive putrefactive changes in the intestine (Emerson (1913), Hawk (1919), Cole (1920), Heitzmann (1921)). Its presence in sprue cases is remarked on by several authorities. Thus Cammidge (1912) found it present in eighty-five per cent. of his cases; Rademaker (1906) mentions it as a diagnostic point, while Castellani and Chalmers (1919) and Byam and Archibald (1923) both refer to its presence; Bahr (1915) notes its occurrence in some of his cases, but regards its presence as of no great significance. Three out of



TABLE

Showing the frequency of indicanuria amongst three hundred and eighty individuals

	Number examined	Percentage positive
Normal individuals ... ..	90	24
Sprue ... ..	4	75
Amoebic Dysentery ... ..	35	94
Bacillary Dysentery ... ..	5	0
Diseases of stomach and small intestine other than the above ...	6	17
Diseases of large intestine other than the above ... ..	23	56
Diseases of the genito-urinary tract ... ..	17	30
Surgical conditions other than the above ... ..	65	15
Lung cases ... ..	62	40
Malaria ... ..	20	50
Various ... ..	53	27

the four cases examined by the present writer were positive. It will be seen from the table that the highest percentage of positive results (94 per cent.) was obtained from amoebic dysentery patients, while the five bacillary dysentery cases examined were all negative. Obviously the number of bacillary cases examined is too small to allow of definite conclusions being drawn, but the marked disparity between the two would suggest that the test may be of some value for differential diagnosis. Quincke and Roos (1893) have drawn attention to the constant presence of indican in the urine of two amoebic dysentery cases, observed by them for respectively eight and eleven months. Ten out of twenty cases of malaria examined gave positive results. The increase of indican in this disease has already been remarked upon by Marchiafava and Bignami (1900) and Craig (1909).

### CONCLUSIONS

Indicanuria occurs in about twenty-five per cent. of apparently normal individuals.

It was present in ninety-four per cent. of amoebic dysentery cases, but was absent from the urine of five cases of bacillary dysentery.

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## MISCELLANEA

### NOTES ON CESTODE PARASITES FROM A DUCK

On July 5th, 1922, A. W. Noel Pillers, Esq., F.R.C.V.S., obtained from York the intestine of a duck, which was found to contain about 300 Cestode parasites.

As the gut had been removed about two days previously, the parasites were partially moribund. The gut was slit open and placed entire in hot Schaudin's preserving fluid, and later, some hundreds of parasites were removed. They were identified as under:—

- (1) *Hymenolepis megalops* (Nitsch, 1829), Par., 1899.

About 20 specimens.

- (2) *Hymenolepis coronula* (Duj., 1845), Cohn, 1901.

About half the collection consisted of this species. The rostellum was armed with about twenty hooks, measuring from  $13\mu$  to  $17\mu$ , of the shape figured by Lühe.

- (3) *Aploparaksis furcigera* (Nitzsch, 1819), Fuhrmann, 1908.

Over one hundred specimens were obtained. Microscopical examination of stained specimens revealed the fact that, whilst nearly all segments contained one testis only, other segments contained two, whilst testes were entirely absent from other segments. This phenomenon was noticed in seven or eight strobilae.

T. SOUTHWELL.

### NOTES ON PARASITIC WORMS FROM THE GOLD COAST

Dr. J. W. S. Macfie sent a number of specimens from Accra, Gold Coast, West Africa, which were identified as follows:—

- (1) *Davainea tetragona* (Molin, 1858), R. Blanchard, 1891.

A large number of specimens from hens.

- (2) A coenurus from the jaws of *Mus rattus*, and also from the pleural cavity of *Cricetomys gambianus*.

This larval form was first described by Turner (1919). The adult form is not known; the hooks bear a close resemblance to those of *C. cerebralis* and of *C. serialis*.

T. SOUTHWELL.

### *CITTOTAENIA LAGORCHESTIS*, LEWIS, 1914

This worm was obtained by Dr. Maplestone from the stomach of an agile wallaby (*Macropus agilis*), taken near Townsville, North Queensland.

T. SOUTHWELL.

### *DAVAINEA LEPTOTRACHELA*, HUNG., 1910

A complete cestode worm from the small intestine of *Turdus semitorquata* (Turtle dove), Pietermaritzburg, Natal, was collected and presented to the School by Mr. Hill, Pietermaritzburg. It proved to be a specimen of the above species.

The suckers are armed and the genital pores irregularly alternate; ovary asymmetrical, situated slightly on the pore side; three or four eggs per capsule, the capsules extending in posterior segments to the lateral margins.

Hungerbühler recorded this species from *Pteroclidurus namaquus* (Grouse).

T. SOUTHWELL.

### *PARAMPHISTOMUM CERVI* IN A HORSE

Some worms collected in May, 1920, from a horse at Tamale, Northern Territories, Gold Coast, and kindly sent to us by Dr. K. B. Allan, proved to be *Paramphistomum cervi*. This record is of interest because, so far as we are able to ascertain, this parasite has not previously been obtained from horses, and is not mentioned as occurring in this host by Maplestone in the 'List of Amphistomes arranged under their hosts' appended to his recent revision of the Amphistomata of Mammals.

J. W. S. MACFIE.



## A NOTE ON *AUCHMEROMYIA LUTEOLA*, FAB.

The bionomics of this fly and its larva the Congo Floor Maggot, first described by Dutton, Todd and Christy (1904), have been very fully described in recent years by Roubaud (1914).



FIG. 1. *Auchmeromyia luteola*. Larva feeding on human skin.  $\times 10$  circ.

The photograph shows the method of feeding which the larva adopts. It stands more or less at right angles to the skin, and has such a firm hold that when the limb is turned over it goes on feeding in a hanging position with equal facility. The feed lasts for as much as an hour in many cases.

The larvae, of which one is photographed feeding on the human arm, were brought alive to England from Sierra Leone at room temperature in sand.

B. BLACKLOCK.

## *ANCYLOSTOMUM CEYLANICUM* IN CATS AND DOGS OF SOUTH INDIA

*Ancylostoma caninum* and *A. ceylanicum* were found in all of five dogs examined at the Veterinary College, Vepery, Madras; in the single cat examined only *A. ceylanicum* was found.

L. S. PARAMESWARA AYYAR.

## THE URINE IN MALARIA

Nephritis as a concomitant of malignant tertian malaria is referred to by most authorities, but its appearance in quartan and simple tertian seems less well known. The following is a record of sixteen consecutive cases of malaria examined at the Liverpool School of Tropical Medicine.

Parasite						Number of cases examined	Number of cases positive
<i>P. malariae</i>	...	...	...	...	...	1	1
<i>P. vivax</i>	...	...	...	...	...	2	2
<i>P. falciparum</i>	...	...	...	...	...	13	7

### PROTOCOLS OF POSITIVE CASES\*

Number of case	Duration of attack	Quinine	Temperature	Parasite	Albumin	Deposit in 5 c.c.'s of centrifuged urine
1	4 days	Yes	103°	<i>P. malariae</i>	+	A few granular casts and renal cells.
2	3 weeks	Yes	105°	<i>P. vivax</i>	+	A few casts and renal cells.
3	3 weeks	Yes	98°	<i>P. vivax</i>	o	A few renal and red cells.
4	3 weeks	Yes	98°	<i>P. falciparum</i>	+	A few hyaline casts and renal cells.
5	2 weeks	Yes	...	<i>P. falciparum</i>	+	Large numbers of granular and hyaline casts and a few renal and red cells.
6	2 months	Yes	98°	<i>P. falciparum</i>	+	Nil.
7	2 months	Yes	98°	<i>P. falciparum</i>	+	A few renal and red cells.
8	1 month	Yes	103°	<i>P. falciparum</i>	o	A few renal and red cells.
9	2 weeks	Yes	98°	<i>P. falciparum</i>	o	Many renal cells; a few red cells.
10	3 weeks	No	98°	<i>P. falciparum</i>	+	A few casts and renal cells.

\* By 'positive' is meant the occurrence of any, or any combination, of the following, albumin, casts, renal epithelium.

R. M. GORDON.

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- A Note on the Action of Lithium Chloride on Mosquito Larvae. By J. W. S. MACFIE, D.Sc., M.B.
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